Fig 1A

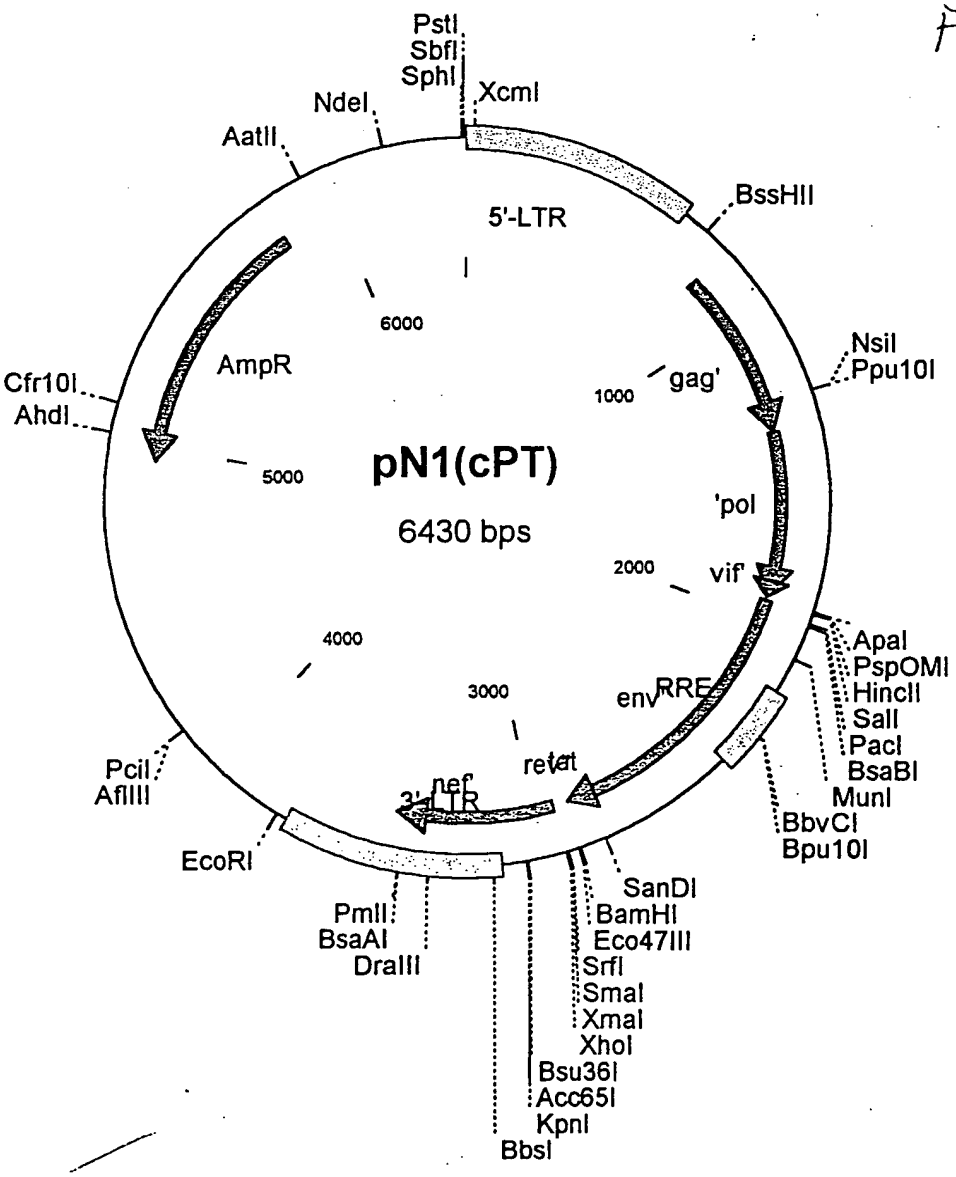

PstI
SbfI
SphI
XcmI
NdeI
AatII
BssHII
5'-LTR
6000
AmpR
1000
gag'
NsiI
Ppu10I
Cfr10I
AhdI
5000
pN1(cPT)
6430 bps
'pol
2000
vif'
4000
ApaI
PspOMI
HincII
SalI
PacI
BsaBI
MunI
3000
env
RRE
rev
tat
nef
3' LTR
PciI
AflIII
BbvCI
Bpu10I
EcoRI
SanDI
PmlI
BamHI
BsaAI
Eco47III
DraIII
SrfI
SmaI
XmaI
XhoI
Bsu36I
Acc65I
KpnI
BbsI

Fig 1B

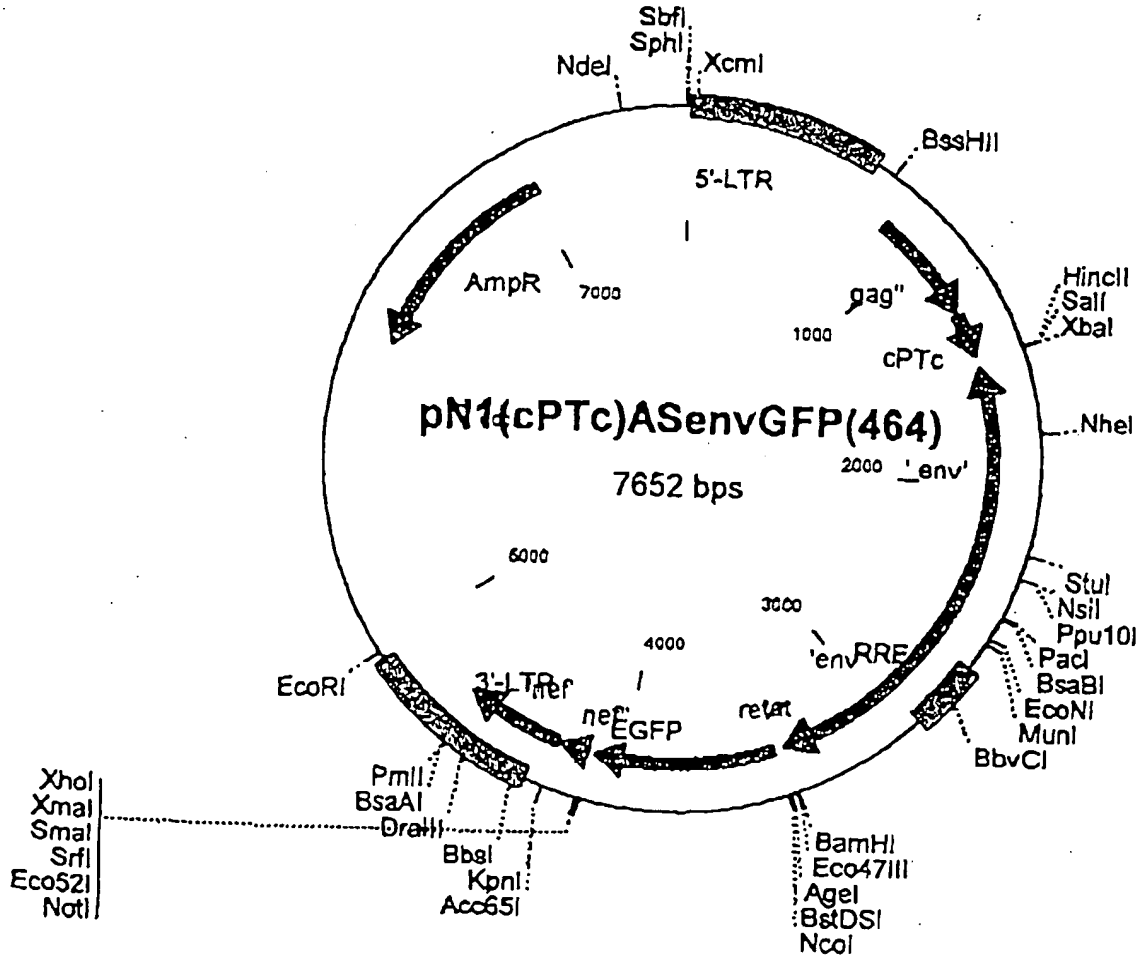

pN1(cPTc)ASenvGFP(464)
7652 bps
5'-LTR
AmpR
7000
gag"
1000
cPTc
2000
'env'
RRE
3000
'env
4000
rev
EGFP
nef"
3'-LTR
6000
EcoRI
SbfI
SphI
NdeI
XcmI
BssHII
HincII
SalI
XbaI
NheI
StuI
NsiI
Ppu10I
PacI
BsaBI
EcoNI
MunI
BbvCI
BamHI
Eco47III
AgeI
BstDSI
NcoI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I

Fig 1C

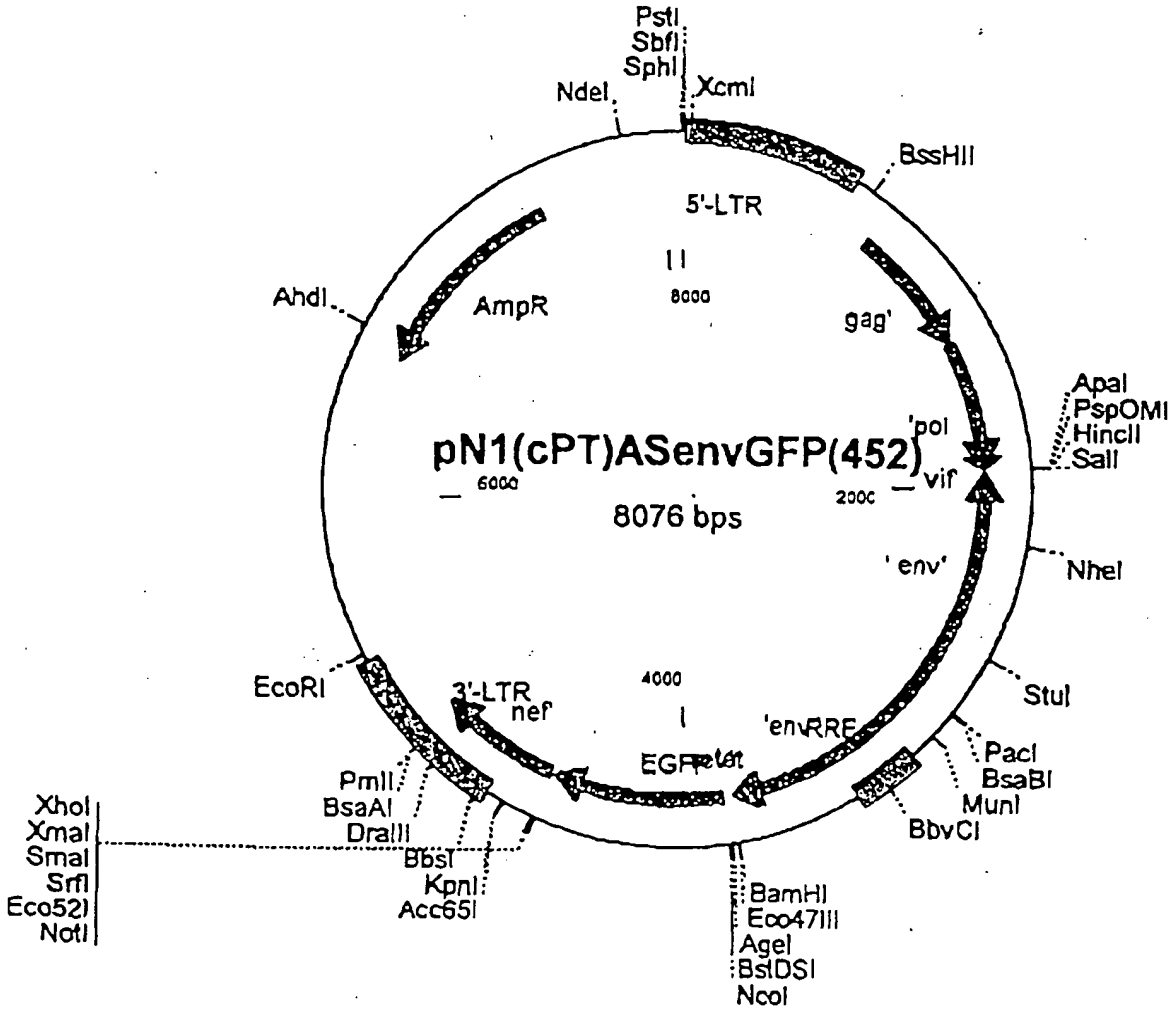

PstI
SbfI
SphI
XcmI
NdeI
BssHII
5'-LTR
AhdI
AmpR
gag'
ApaI
PspOMI
HincII
SalI
pN1(cPT)ASenvGFP(452)
8076 bps
'pol
vif
'env'
NheI
EcoRI
3'-LTR
nef
'envRRE
StuI
PacI
BsaBI
MunI
BbvCI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BamHI
Eco47III
AgeI
BstDSI
NcoI

Fig 1D

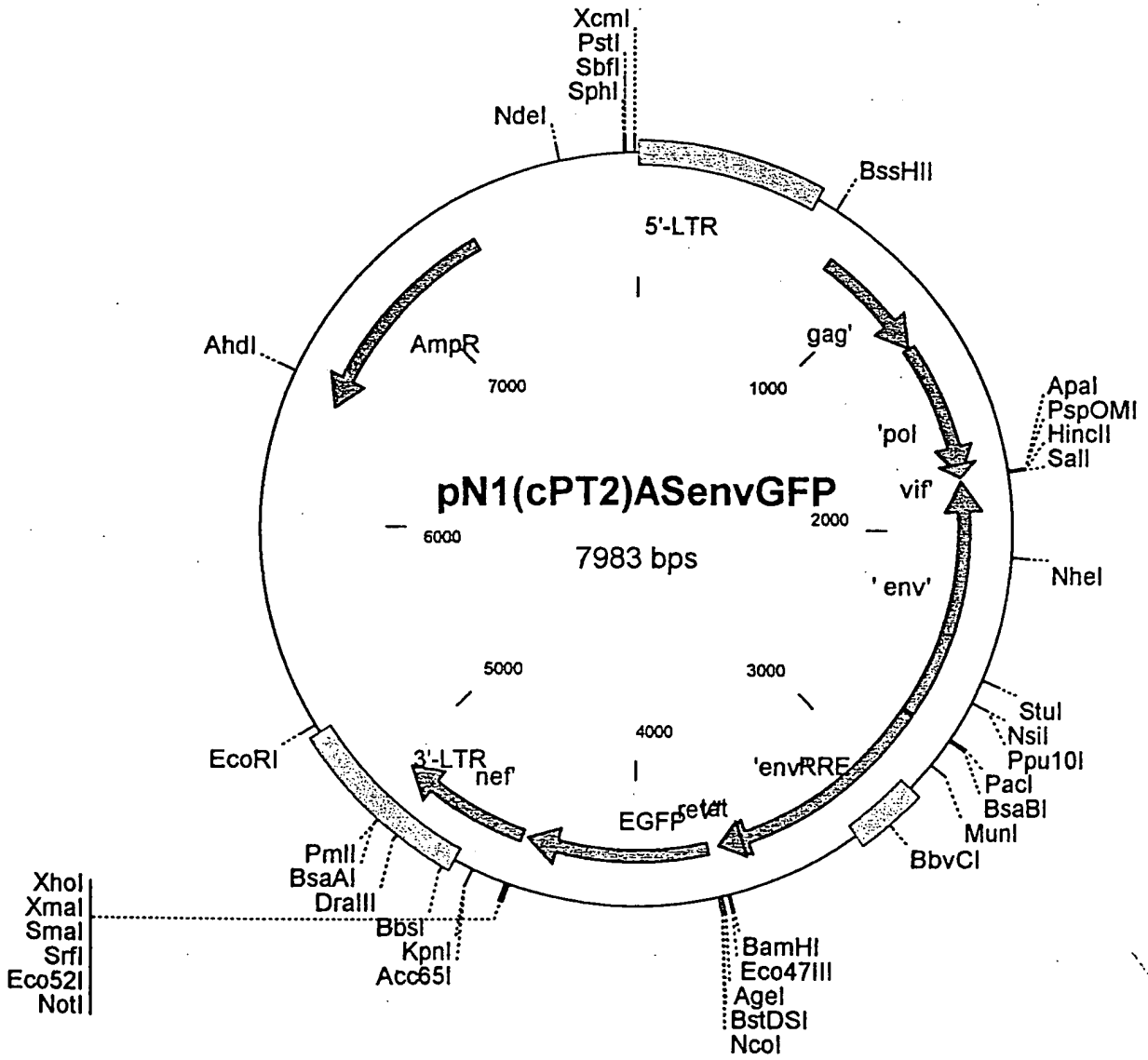

pN1(cPT2)ASenvGFP
7983 bps
5'-LTR
gag'
'pol
vif'
' env'
'envRRE
retet
EGFP
3'-LTR
nef'
AmpR
1000
2000
3000
4000
5000
6000
7000
XcmI
PstI
SbfI
SphI
NdeI
BssHII
ApaI
PspOMI
HincII
SalI
NheI
StuI
NsiI
Ppu10I
PacI
BsaBI
MunI
BbvCI
BamHI
Eco47III
AgeI
BstDSI
NcoI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
EcoRI
AhdI

Fig 1E

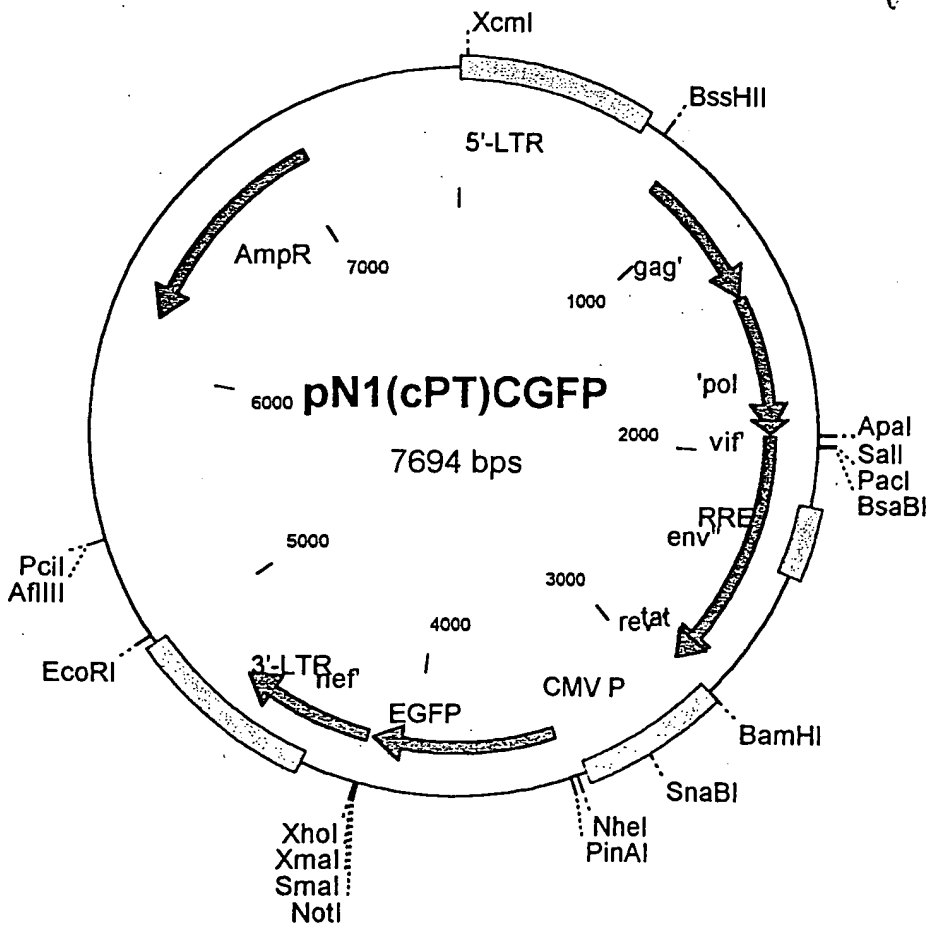

XcmI
BssHII
5'-LTR
1
AmpR
7000
gag'
1000
6000
pN1(cPT)CGFP
7694 bps
'pol
2000
vif'
ApaI
SalI
PacI
BsaBI
RRE
env'
5000
3000
rev
tat
PciI
AflIII
4000
EcoRI
3'-LTR
nef'
CMV P
EGFP
BamHI
SnaBI
NheI
PinAI
XhoI
XmaI
SmaI
NotI

Fig 1F

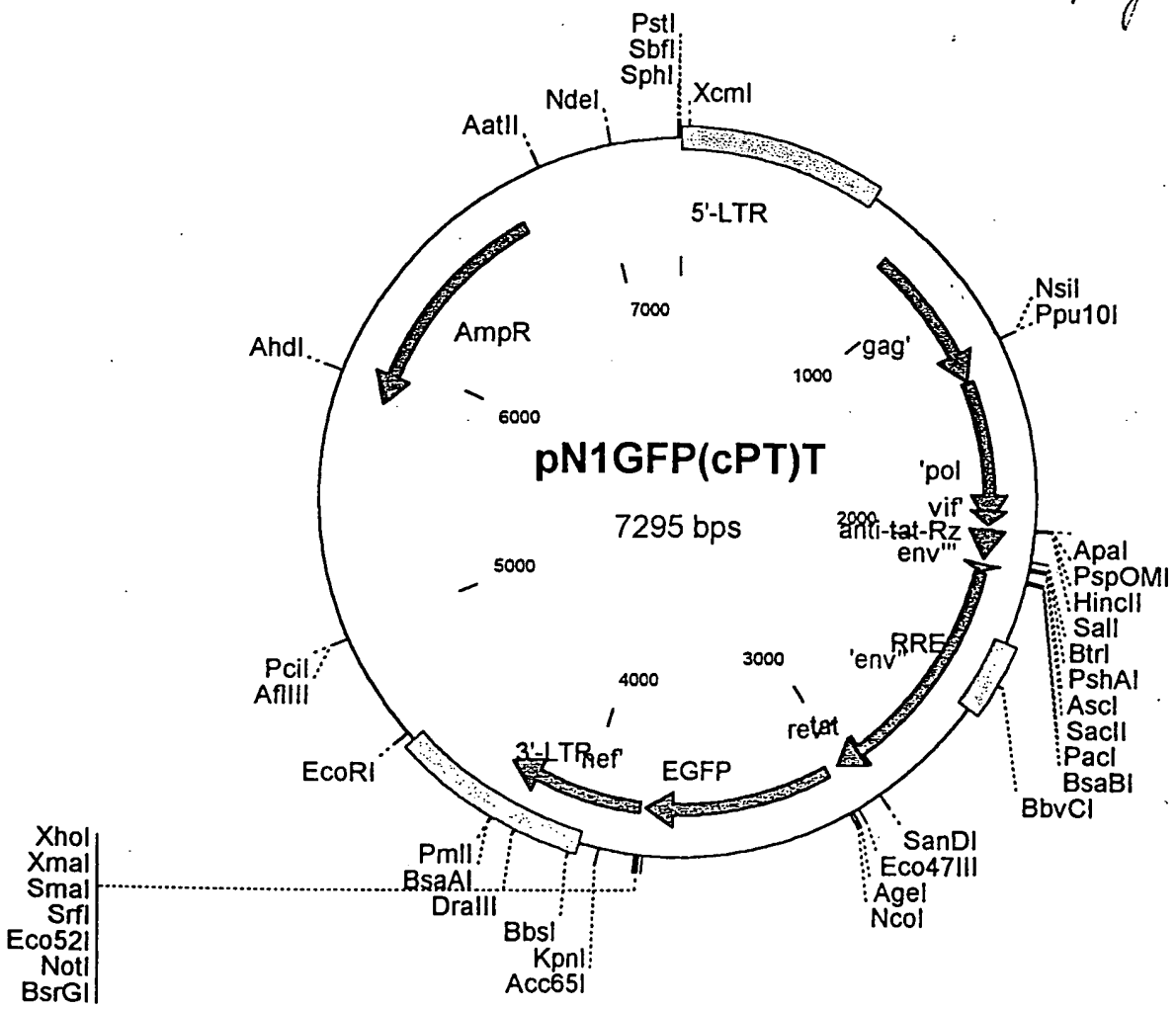

pN1GFP(cPT)T
7295 bps
5'-LTR
gag'
'pol
vif'
anti-tat-Rz
env'''
RRE
'env''
rev
tat
EGFP
nef'
3'-LTR
AmpR
1000
2000
3000
4000
5000
6000
7000
PstI
SbfI
SphI
XcmI
NdeI
AatII
NsiI
Ppu10I
ApaI
PspOMI
HincII
SalI
BtrI
PshAI
AscI
SacII
PacI
BsaBI
BbvCI
SanDI
Eco47III
AgeI
NcoI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
EcoRI
PciI
AflIII
AhdI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI

Fig 1G

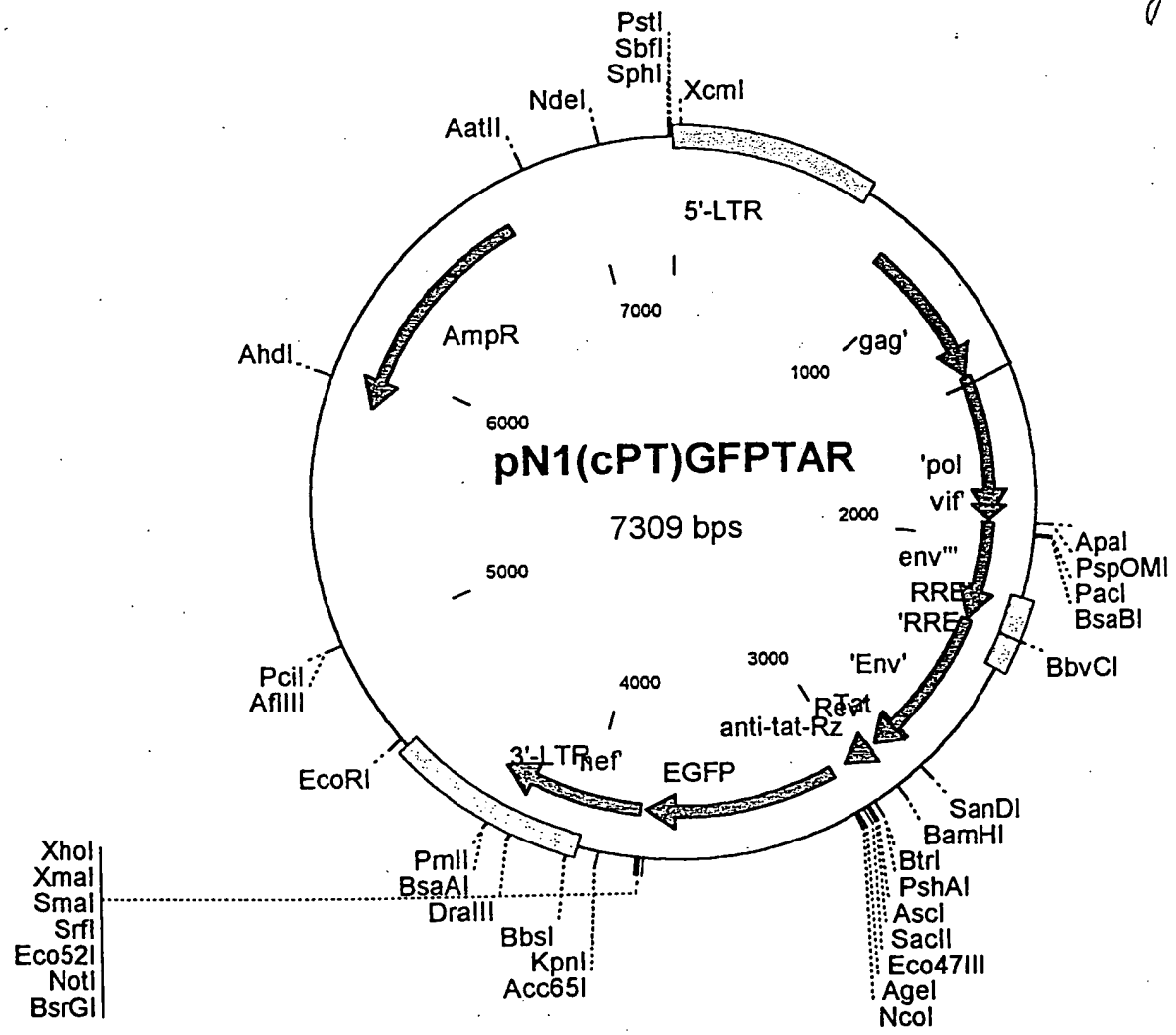

pN1(cPT)GFPTAR
7309 bps
5'-LTR
7000
1000
2000
3000
4000
5000
6000
AmpR
'gag'
'pol
vif'
env'''
RRE
'RRE
'Env'
Rev
Tat
anti-tat-Rz
nef'
EGFP
3'-LTR
PstI
SbfI
SphI
XcmI
NdeI
AatII
AhdI
ApaI
PspOMI
PacI
BsaBI
BbvCI
SanDI
BamHI
BtrI
PshAI
AscI
SacII
Eco47III
AgeI
NcoI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
EcoRI
PciI
AflIII
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI

Fig 1H

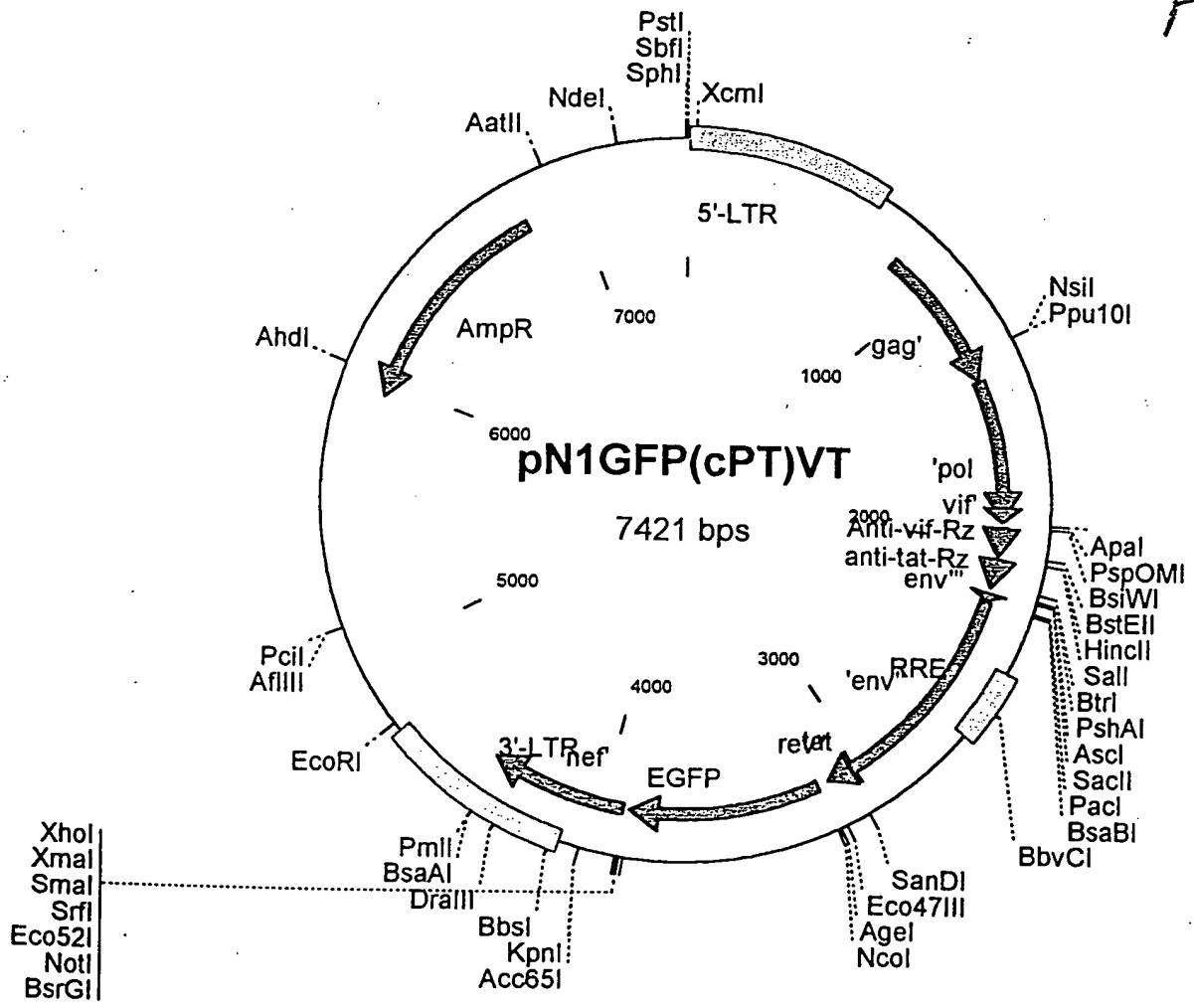
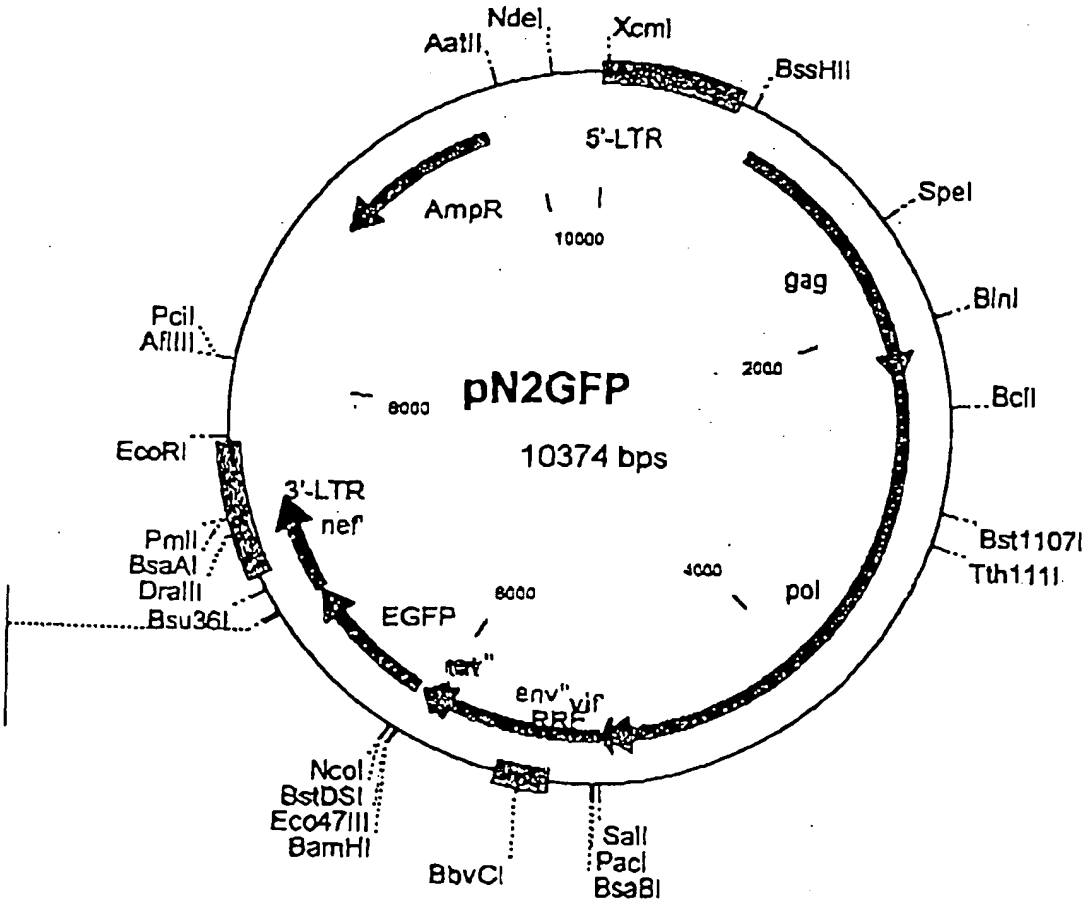

pN1GFP(cPT)VT
7421 bps
1
1000
2000
3000
4000
5000
6000
7000
5'-LTR
'gag'
'pol
vif'
Anti-vif-Rz
anti-tat-Rz
env'''
'env
RRE
rev
3'-LTR
nef'
EGFP
AmpR
PstI
SbfI
SphI
XcmI
NdeI
AatII
NsiI
Ppu10I
AhdI
ApaI
PspOMI
BsiWI
BstEII
HincII
SalI
BtrI
PshAI
AscI
SacII
PacI
BsaBI
BbvCI
PciI
AflIII
EcoRI
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
BsrGI
PmlI
BsaAI
DraIII
BbsI
KpnI
Acc65I
SanDI
Eco47III
AgeI
NcoI

Fig 1I


pN2GFP
10374 bps
5'-LTR
gag
pol
env
vif
RRE
rev
tat
EGFP
nef
3'-LTR
AmpR
2000
4000
6000
8000
10000
NdeI
AatII
XcmI
BssHII
SpeI
BlnI
BclI
Bst1107I
Tth111I
PciI
AflIII
EcoRI
PmlI
BsaAI
DraIII
Bsu36I
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
NcoI
BstDSI
Eco47III
BamHI
BbvCI
SalI
PacI
BsaBI

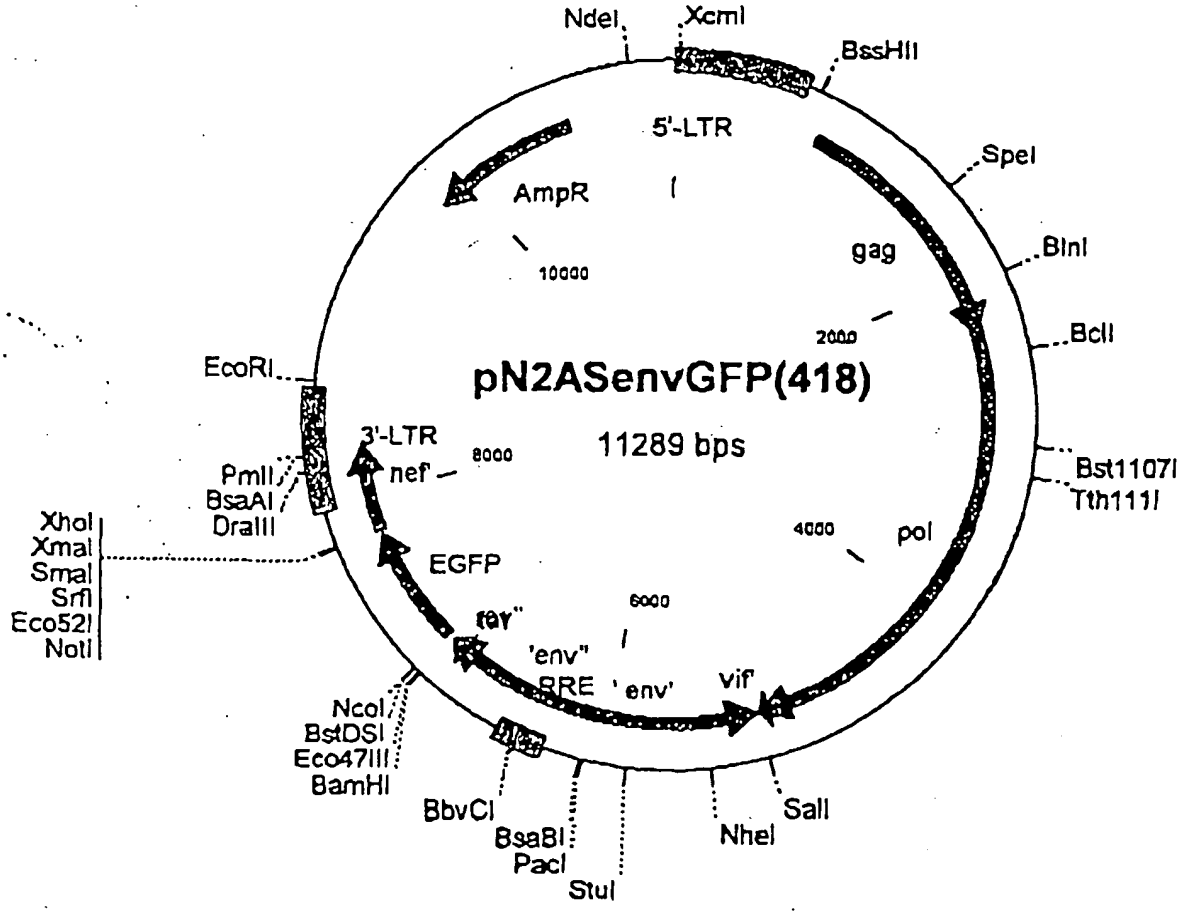
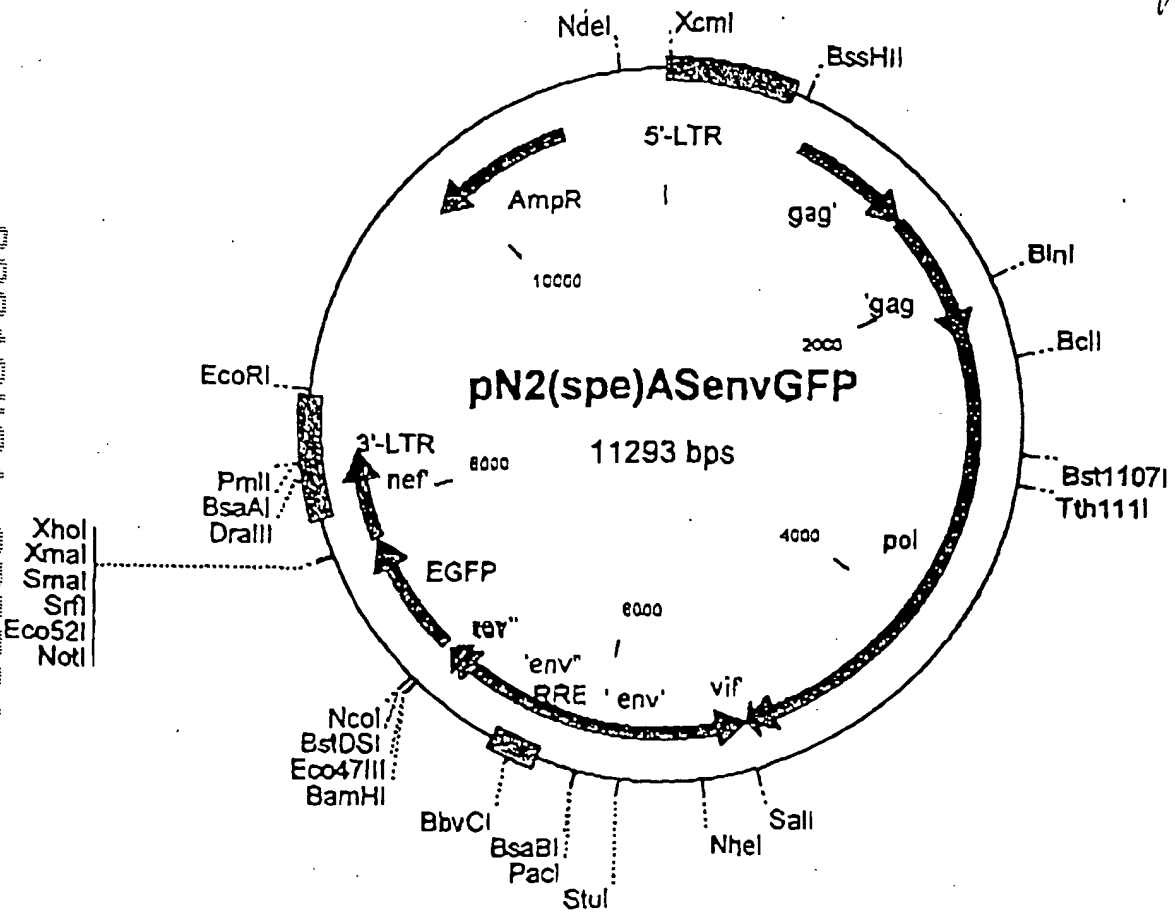

NdeI
XcmI
BssHII
5'-LTR
SpeI
AmpR
gag
BlnI
10000
2000
BclI
EcoRI
pN2ASenvGFP(418)
11289 bps
3'-LTR
8000
Bst1107I
Tth111I
PmlI
BsaAI
DraIII
nef
4000
pol
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
EGFP
6000
rev
'env"
RRE
'env'
vif
NcoI
BstDSI
Eco47III
BamHI
BbvCI
BsaBI
PacI
StuI
NheI
SalI

Fig 1J

Fig 1K


NdeI
XcmI
BssHII
5'-LTR
AmpR
gag'
BlnI
10000
'gag
2000
BclI
EcoRI
pN2(spe)ASenvGFP
11293 bps
3'-LTR
8000
nef
PmlI
BsaAI
DraIII
Bst1107I
Tth111I
4000
pol
XhoI
XmaI
SmaI
SrfI
Eco52I
NotI
EGFP
6000
tev
'env'
RRE
'env'
vif
NcoI
BstDSI
Eco47III
BamHI
BbvCI
BsaBI
PacI
StuI
NheI
SalI

A +105 GTGTGCCCGTCTG +117

B .....AC...

A +118 TTGTGTGACTCTG +130

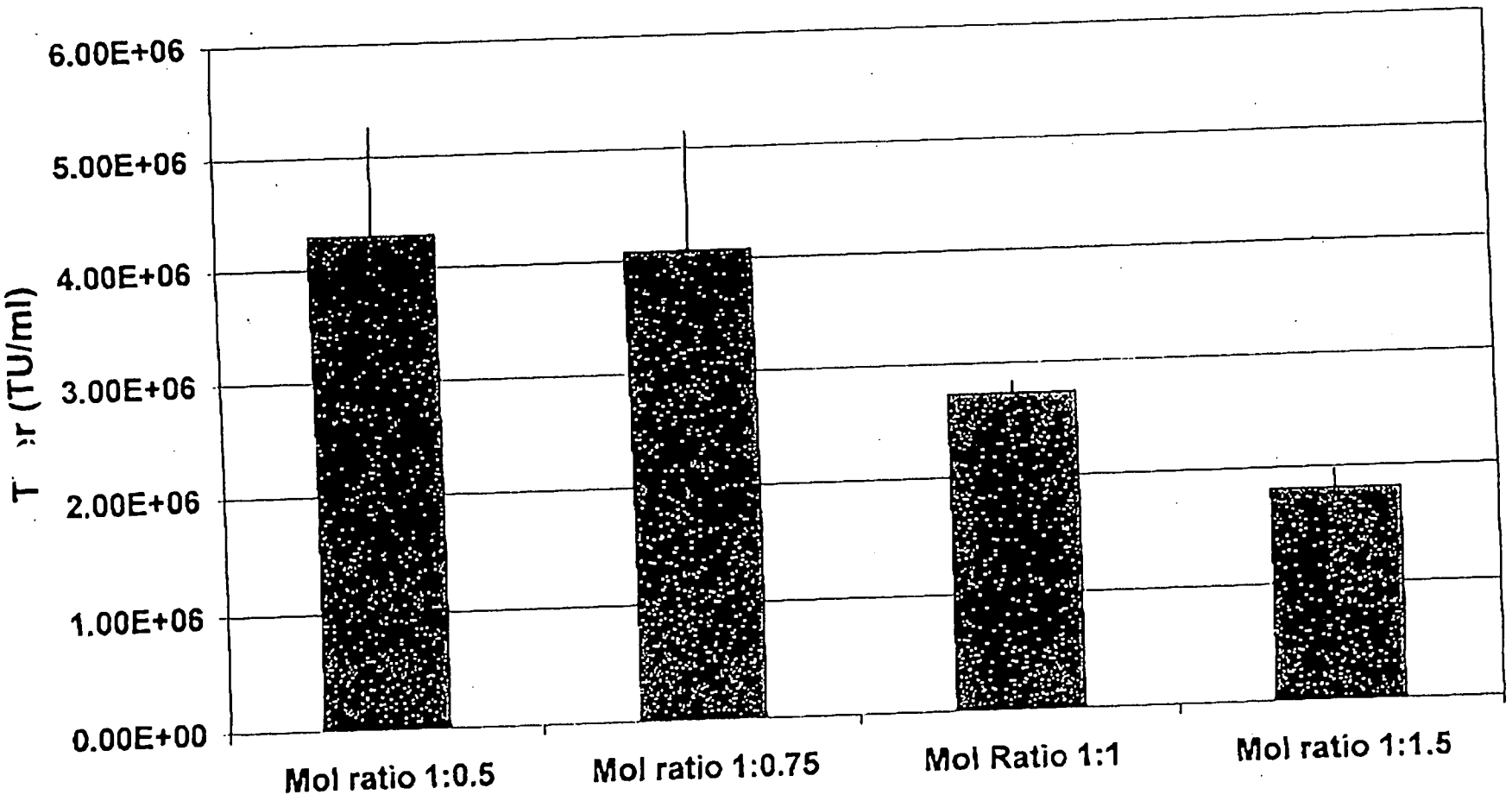
B .....

A +131 GTAACTAGAGATC +143

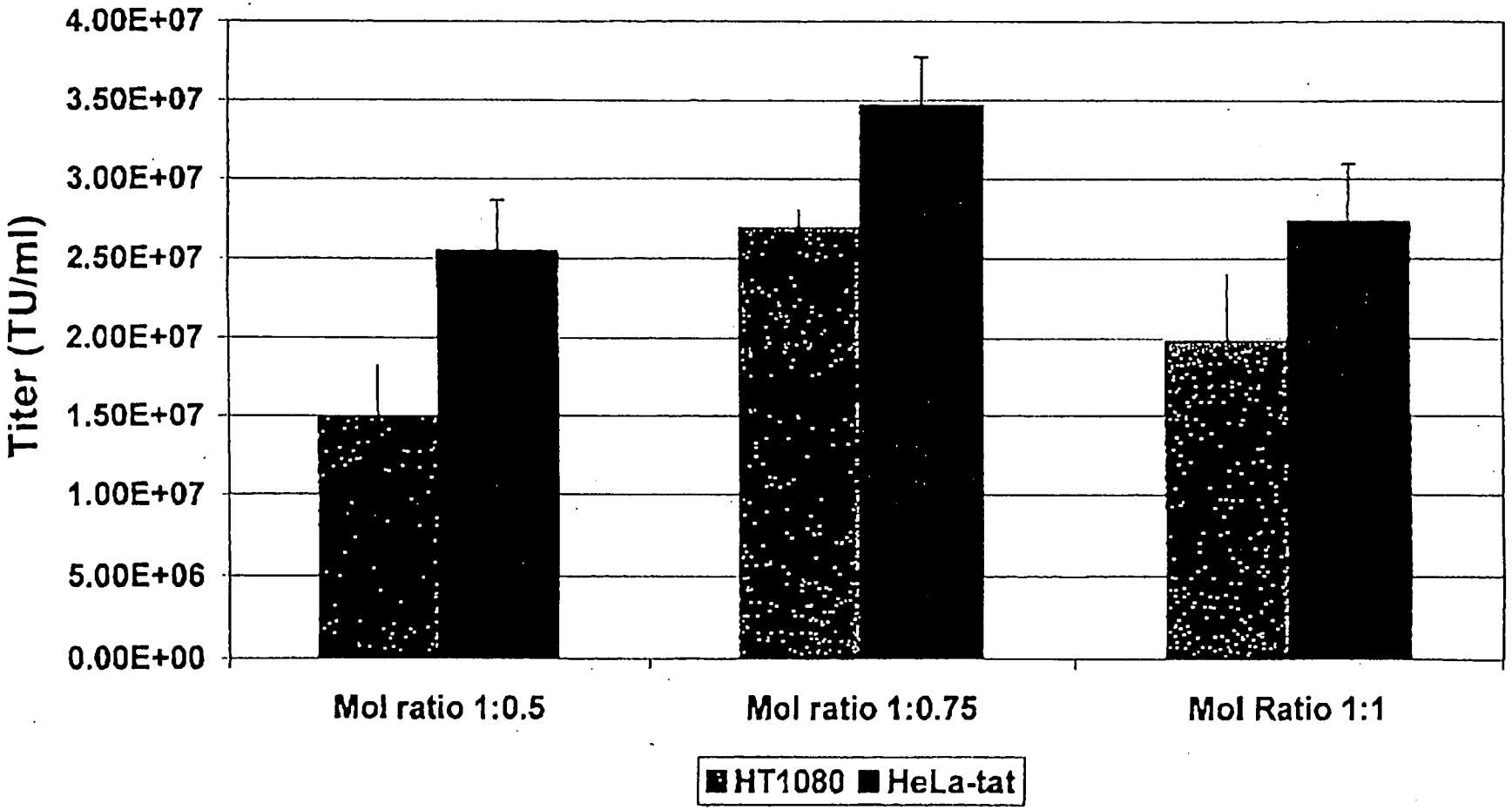
B .C.G.....A.

FIG. 2

Ratio Optimization for pN1(cPTC)ASenvGFP Vector

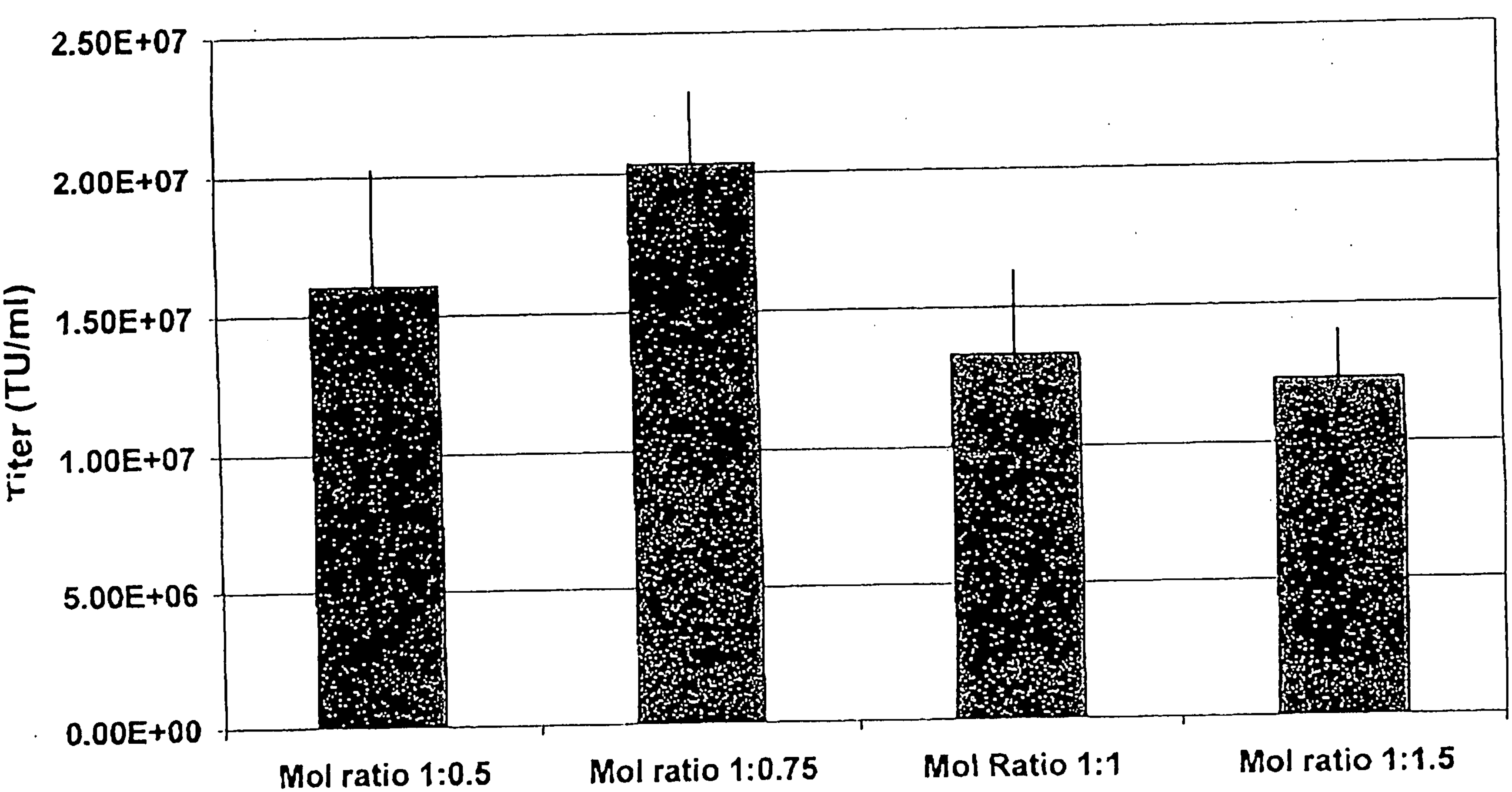


Ratio Optimization for pN1(cPT)GFP Vectors



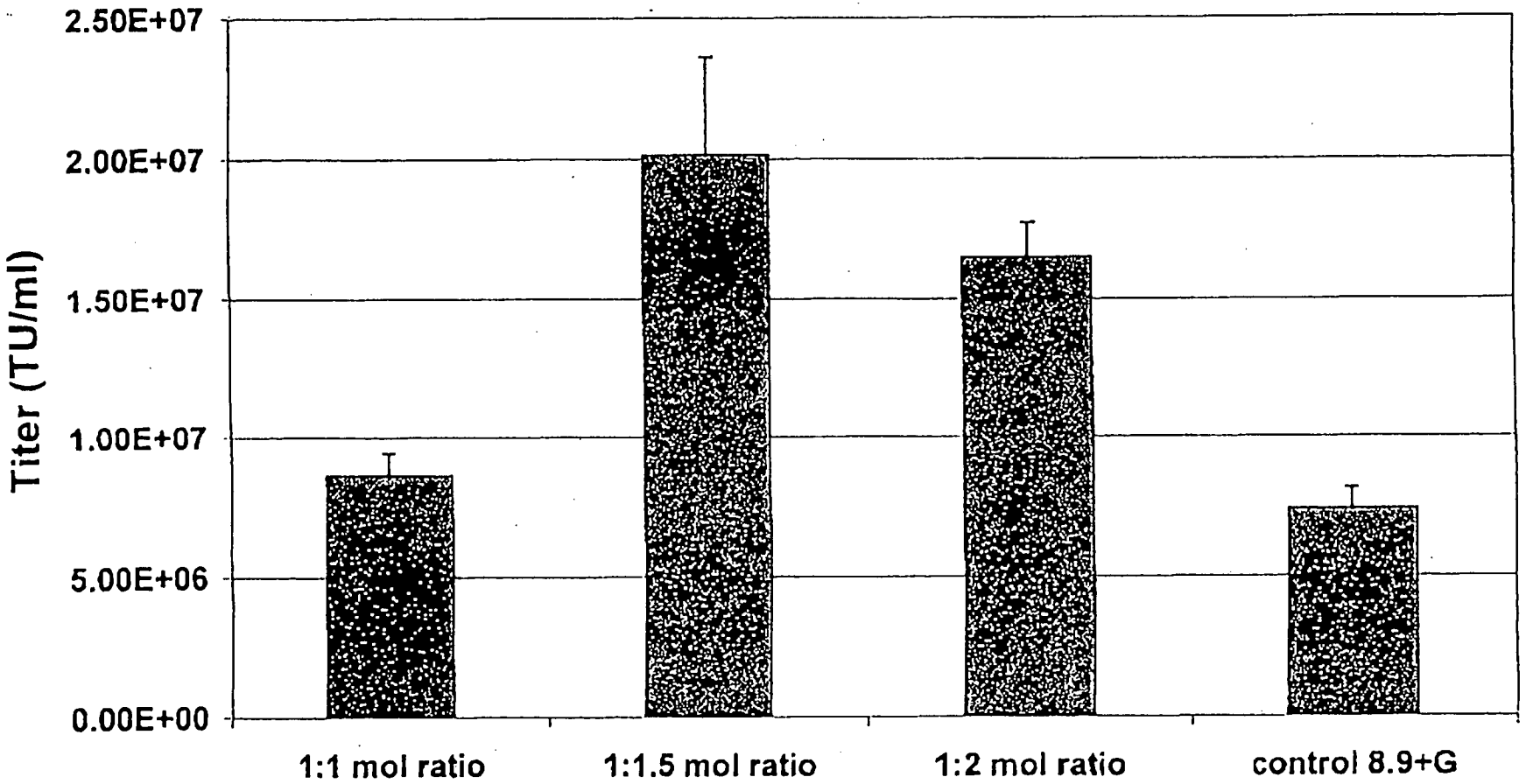
3C

Ratio Optimization for pN1(cPT2)ASenvGFP Vector



3D

Best Vector to Packaging Ratio for pN1cGFP Vector



3E

Optimiztion of vector to packaging ratio for pN2cGFP

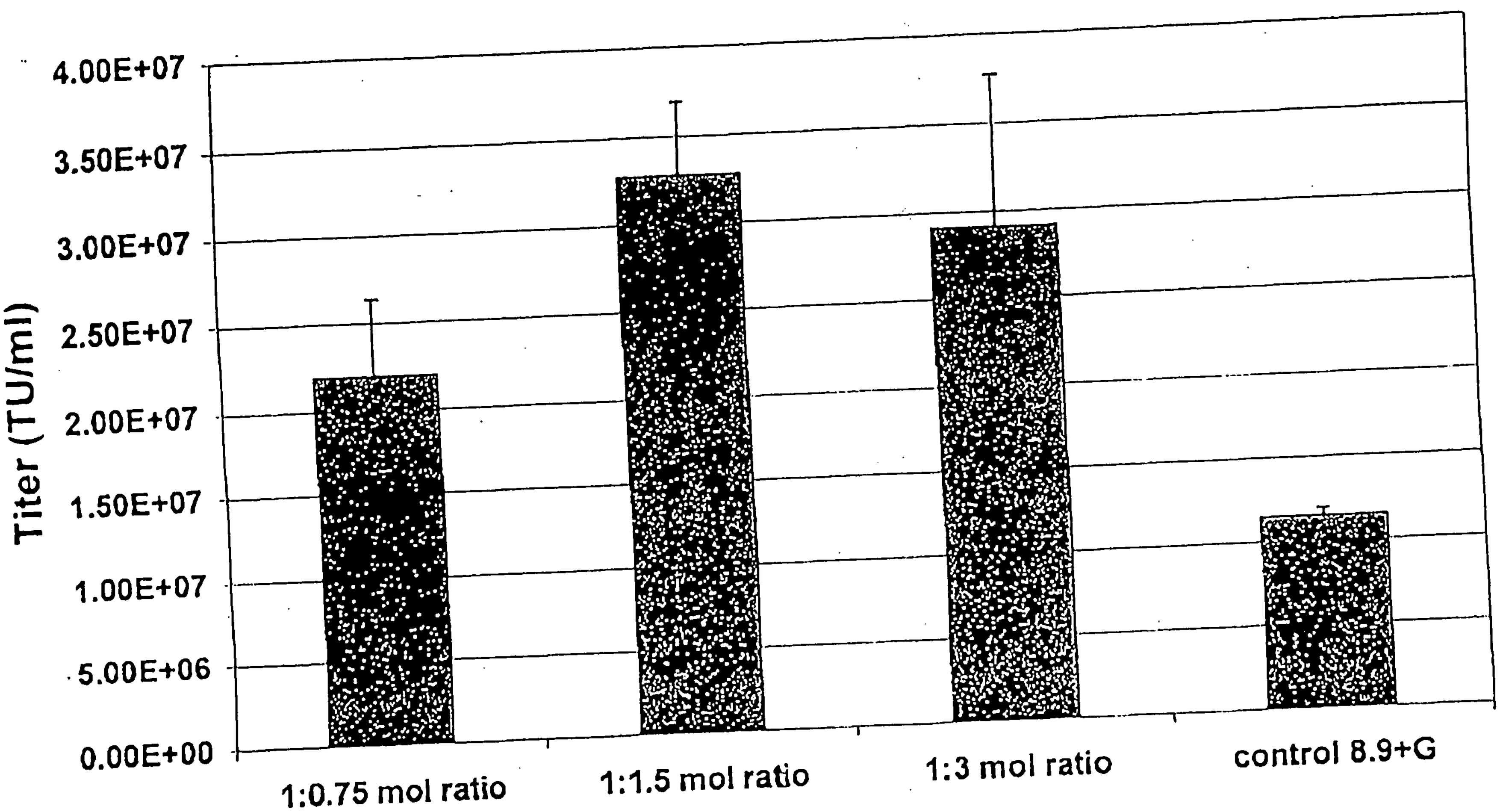


Fig 4A

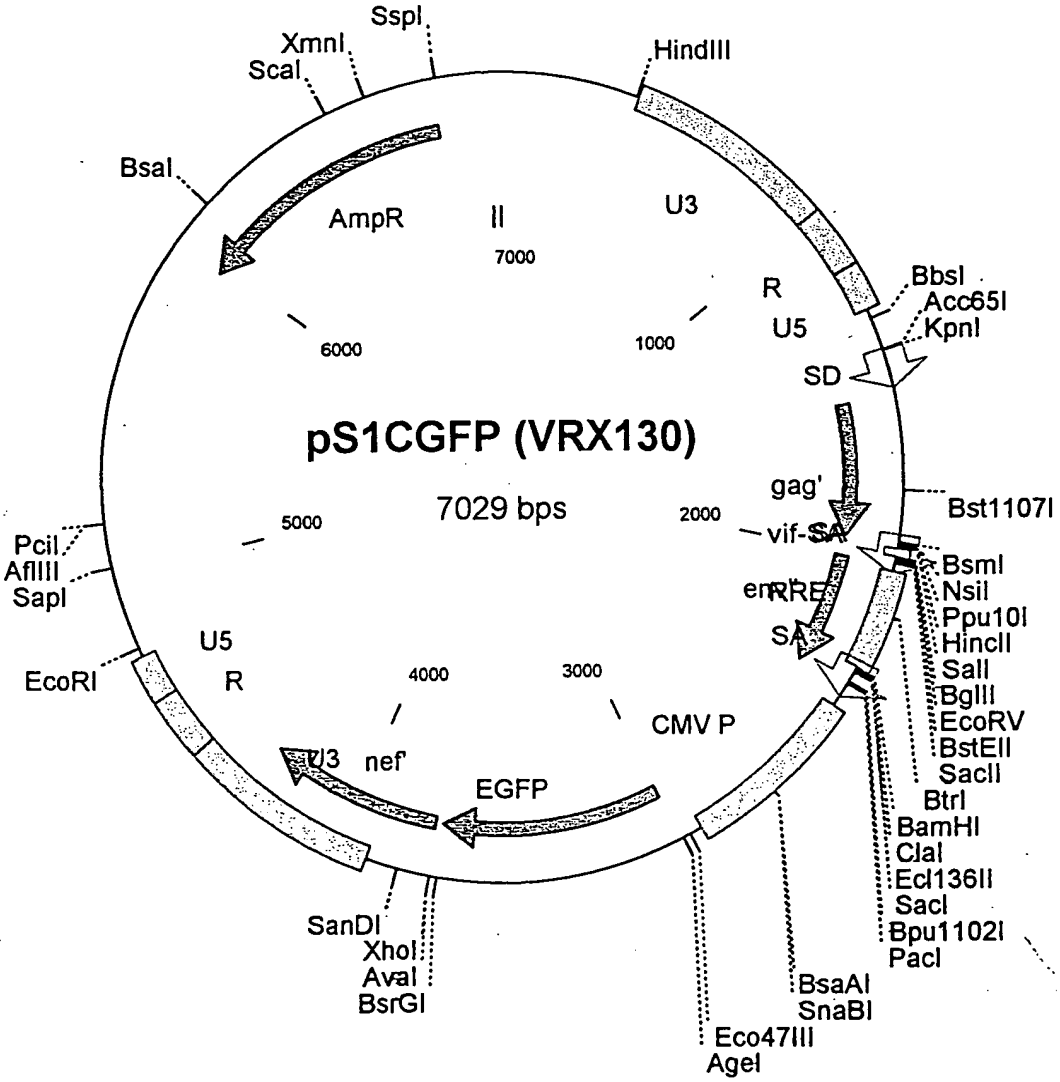
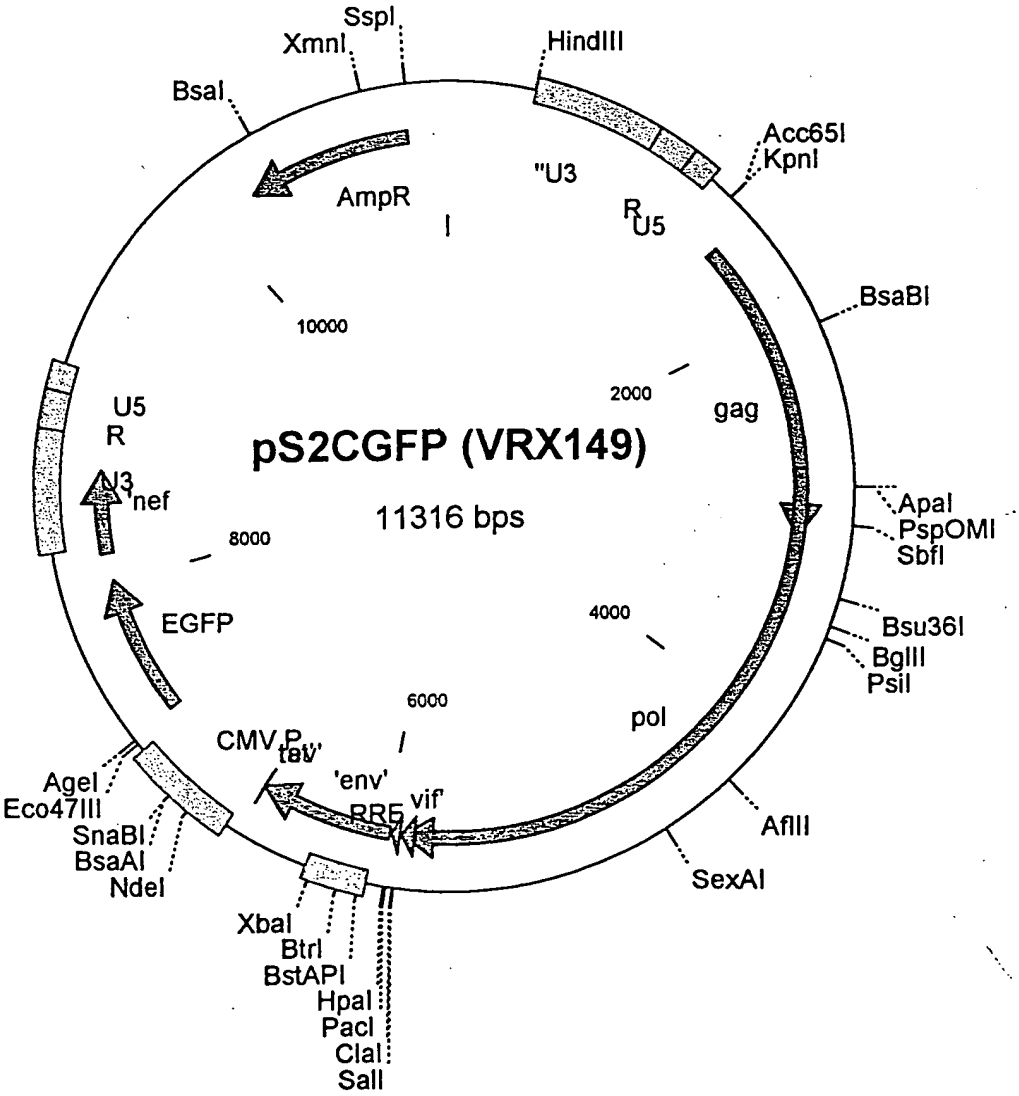
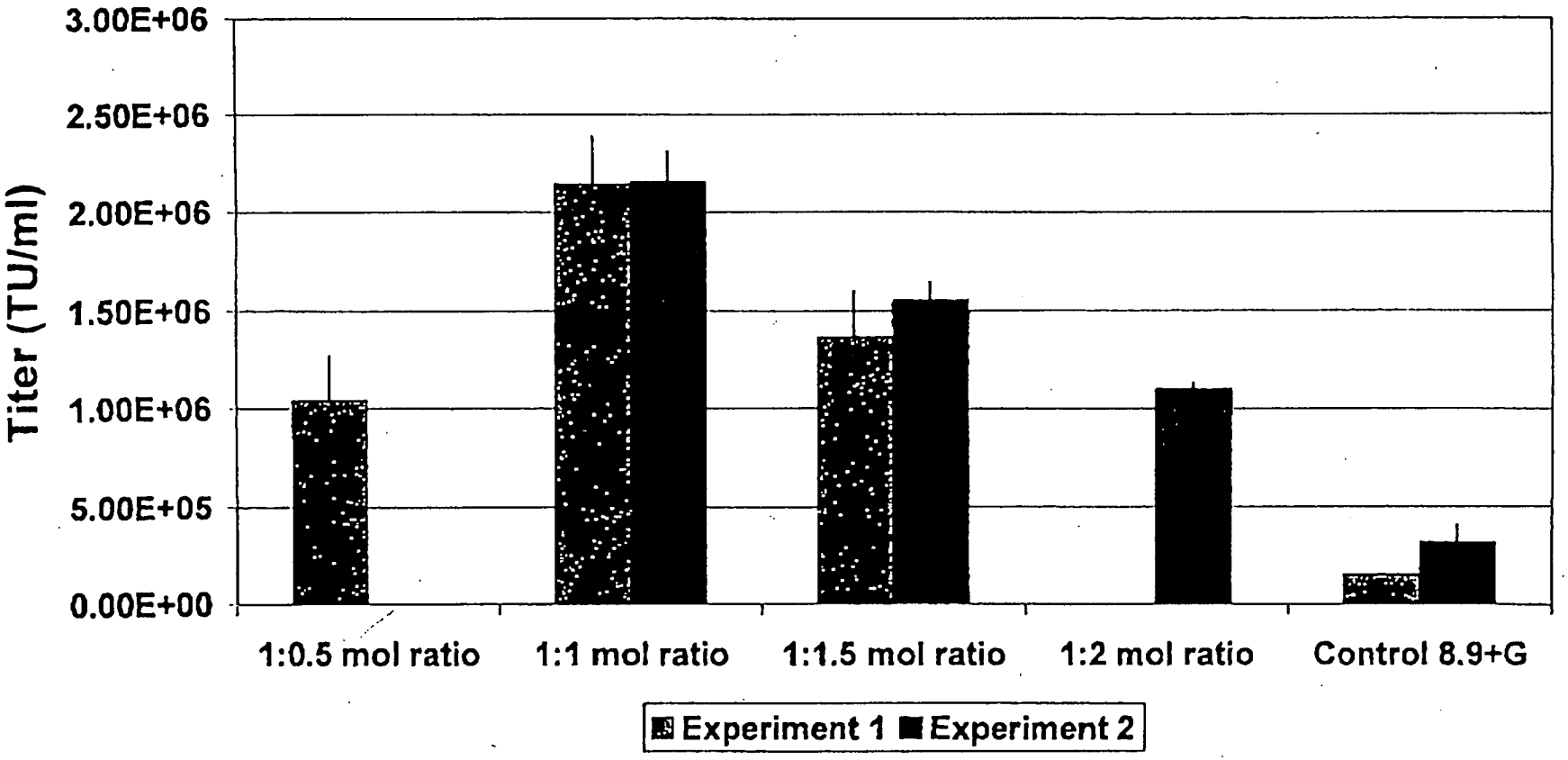

pS1CGFP (VRX130)
7029 bps
SspI
XmnI
ScaI
HindIII
BsaI
AmpR
ll
U3
7000
R
U5
1000
6000
BbsI
Acc65I
KpnI
SD
gag'
2000
Bst1107I
5000
vif-SA
BsmI
NsiI
Ppu10I
HincII
SalI
BglII
EcoRV
BstEII
SacII
BtrI
BamHI
ClaI
Ecl136II
SacI
Bpu1102I
PacI
PciI
AflIII
SapI
U5
R
EcoRI
4000
3000
CMV P
U3
nef
EGFP
SanDI
XhoI
AvaI
BsrGI
BsaAI
SnaBI
Eco47III
AgeI

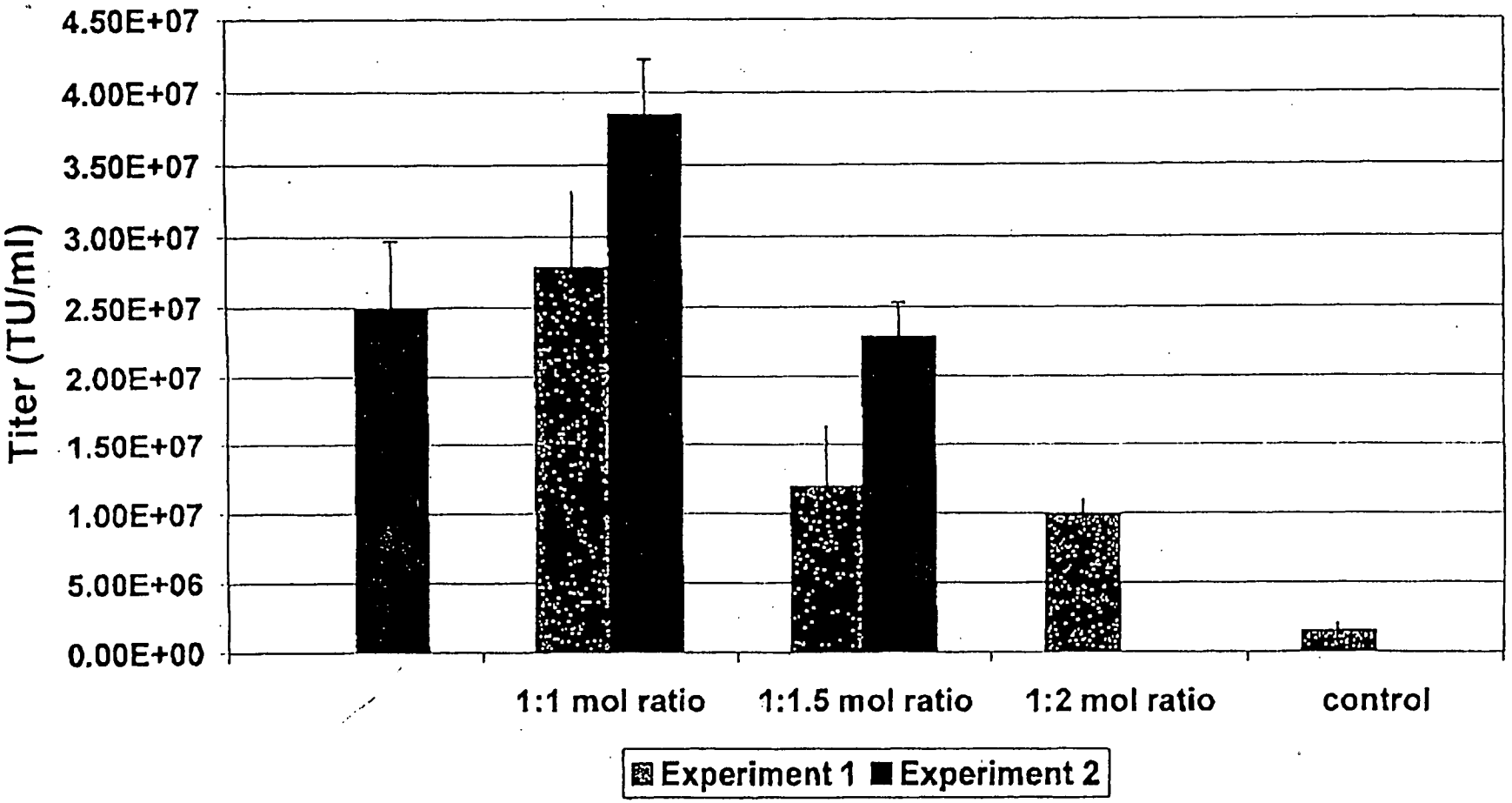
Fig 4B


pS2CGFP (VRX149)
11316 bps
SspI
XmnI
HindIII
BsaI
Acc65I
KpnI
AmpR
"U3
R
U5
BsaBI
10000
2000
gag
U5
R
U3
nef
8000
ApaI
PspOMI
SbfI
EGFP
4000
Bsu36I
BglII
PsiI
6000
pol
CMV P
tat
'env'
vif'
RRE
AgeI
Eco47III
SnaBI
BsaAI
NdeI
AflII
SexAI
XbaI
BtrI
BstAPI
HpaI
PacI
ClaI
SalI

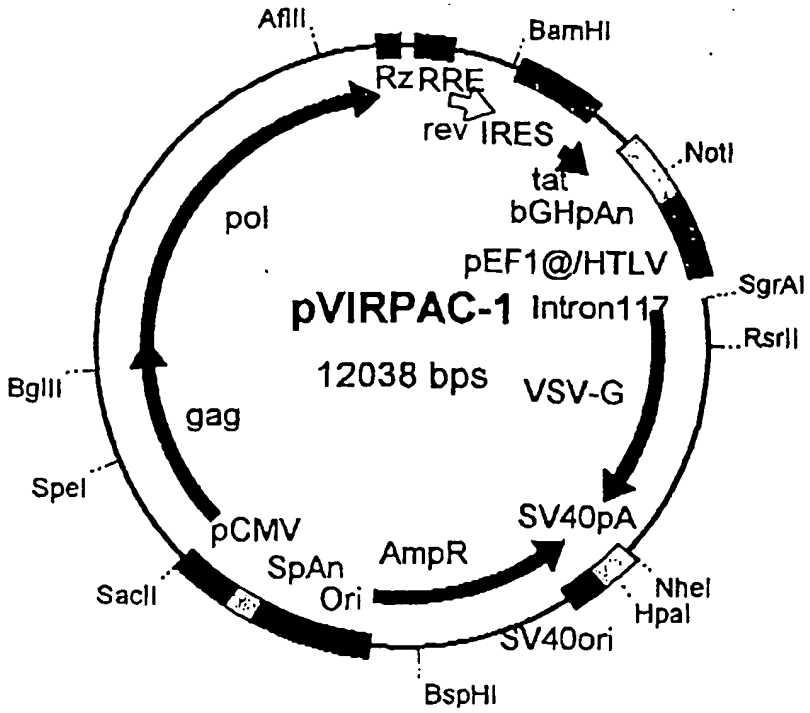
Ratio Optimization for Packaging of pS1cGFP vectors.



Optimiztion of vector to packaging ratio for pS2cGFP



Packaging Construct



New features:

- First 42 nt of gag are degenerated.
- Tat and rev represented as cDNA.
- First 208 nt of rev and last 183 nt of tat are degenerated.
- RRE from HIV-2 is used instead of HIV-1 RRE.

These features eliminate almost any homology with the vector plasmid, make system safer.

- Anti-U5 ribozyme is expressed within gag/pol/RRE cassette, further improving safety.
- Gag/pol/rev/tat/RRE cassette and VSV-G expressed from the same plasmid. This feature may enhance packaging efficiency and titers of the vectors.

Fig. 6B Packaging Plasmid for Second Generation Vectors

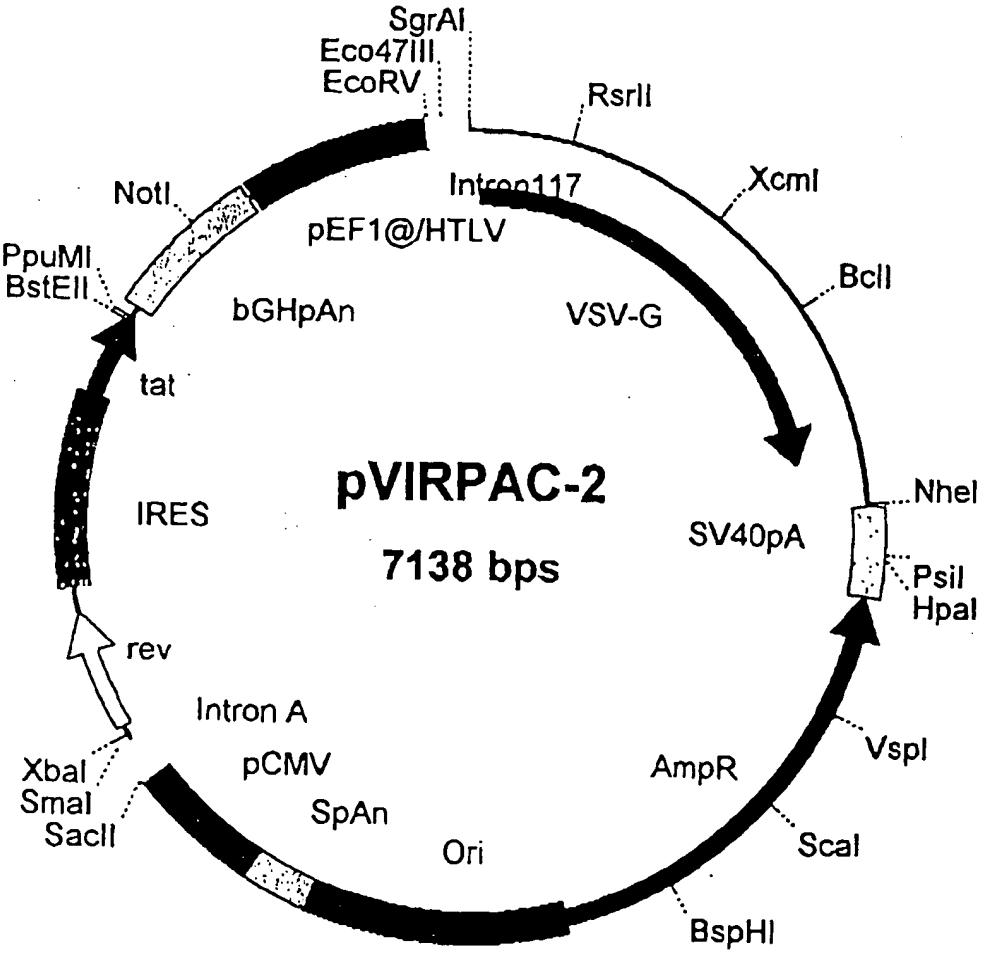


Fig. 6C Packaging Plasmid for First Generation Vectors

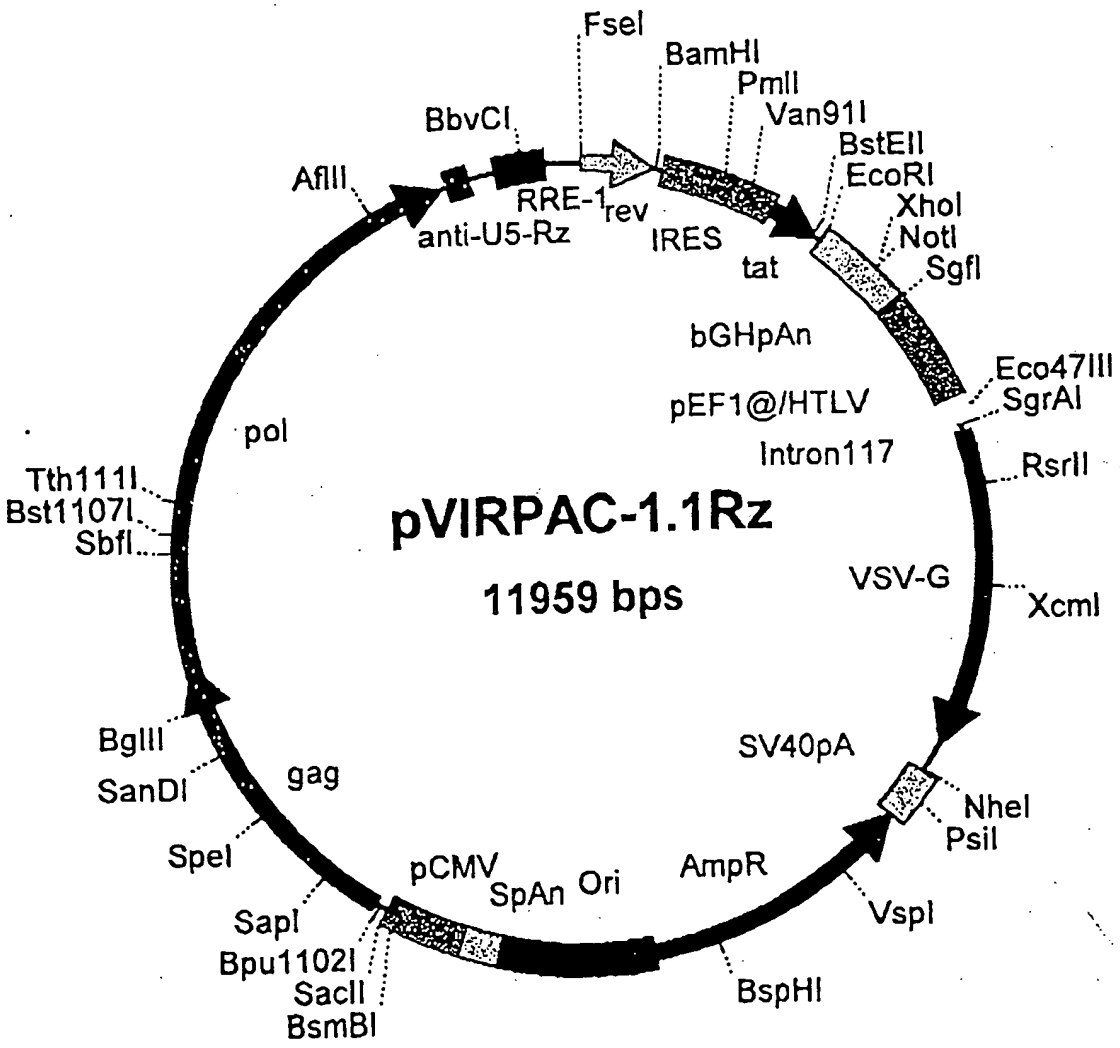


Fig 6D

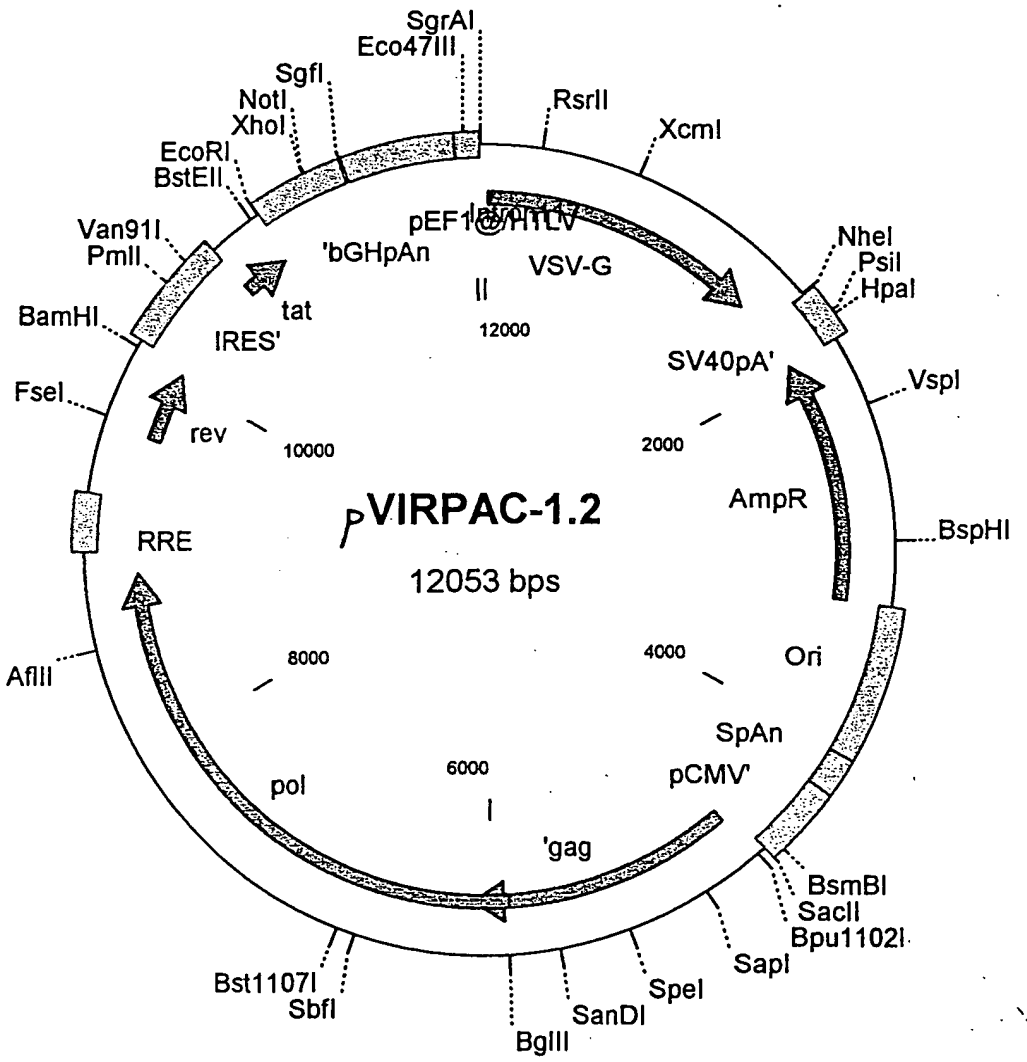

pVIRPAC-1.2
12053 bps
SgrAI
Eco47III
SgfI
NotI
XhoI
EcoRI
BstEII
Van91I
PmlI
BamHI
FseI
AflII
RRE
rev
IRES'
tat
'bGHpAn
pEF1@HTLV
VSV-G
RsrII
XcmI
NheI
PsiI
HpaI
VspI
BspHI
Ori
SpAn
pCMV'
'gag
pol
Bst1107I
SbfI
BglII
SanDI
SpeI
SapI
BsmBI
SacII
Bpu1102I
2000
4000
6000
8000
10000
12000
SV40pA'
AmpR

Fig 6E

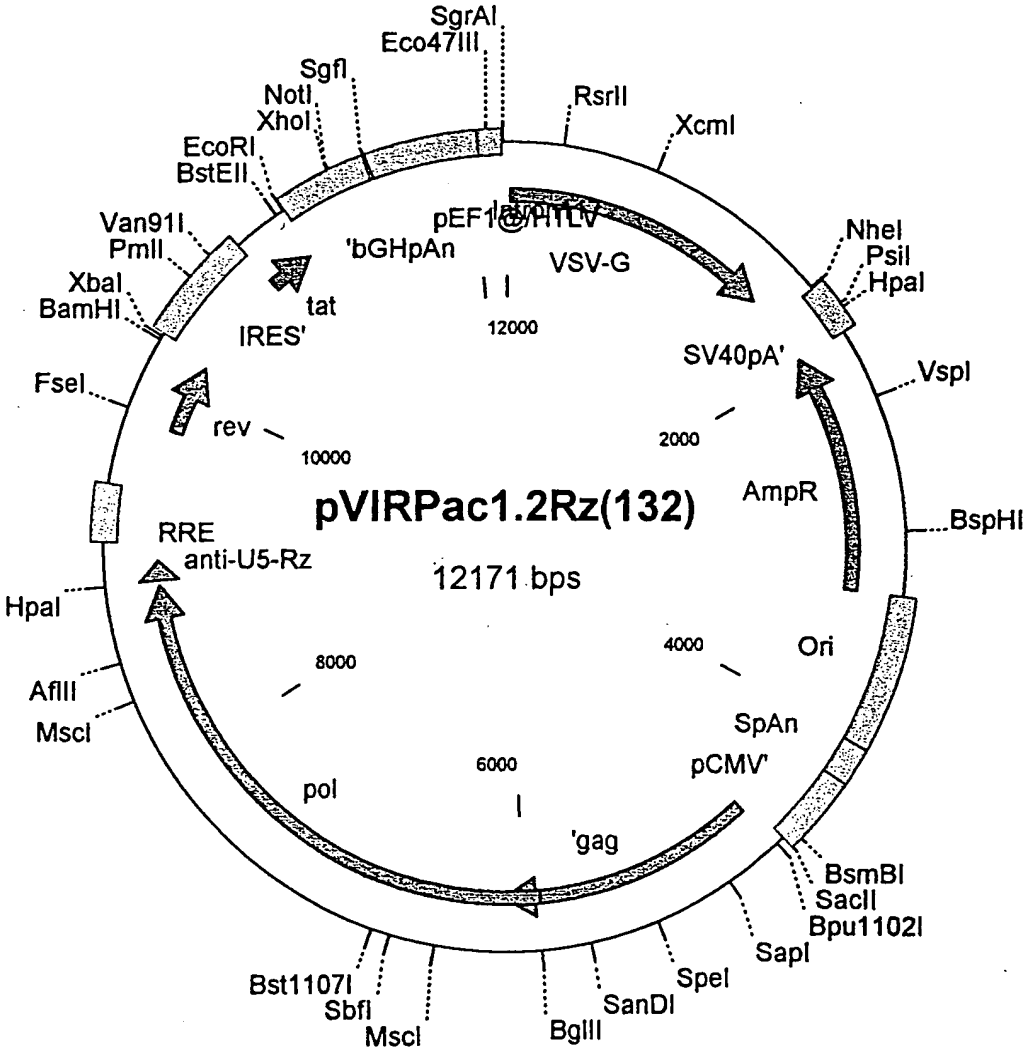

pVIRPac1.2Rz(132)
12171 bps
SgrAI
Eco47III
SgfI
NotI
XhoI
EcoRI
BstEII
Van91I
PmlI
XbaI
BamHI
FseI
HpaI
AflII
MscI
Bst1107I
SbfI
MscI
BglII
SanDI
SpeI
SapI
BsmBI
SacII
Bpu1102I
BspHI
VspI
NheI
PsiI
HpaI
RsrII
XcmI
pEF1α/HTLV
'bGHpAn
VSV-G
tat
IRES'
rev
RRE
anti-U5-Rz
pol
'gag
pCMV'
SpAn
Ori
AmpR
SV40pA'
2000
4000
6000
8000
10000
12000

Fig 6F

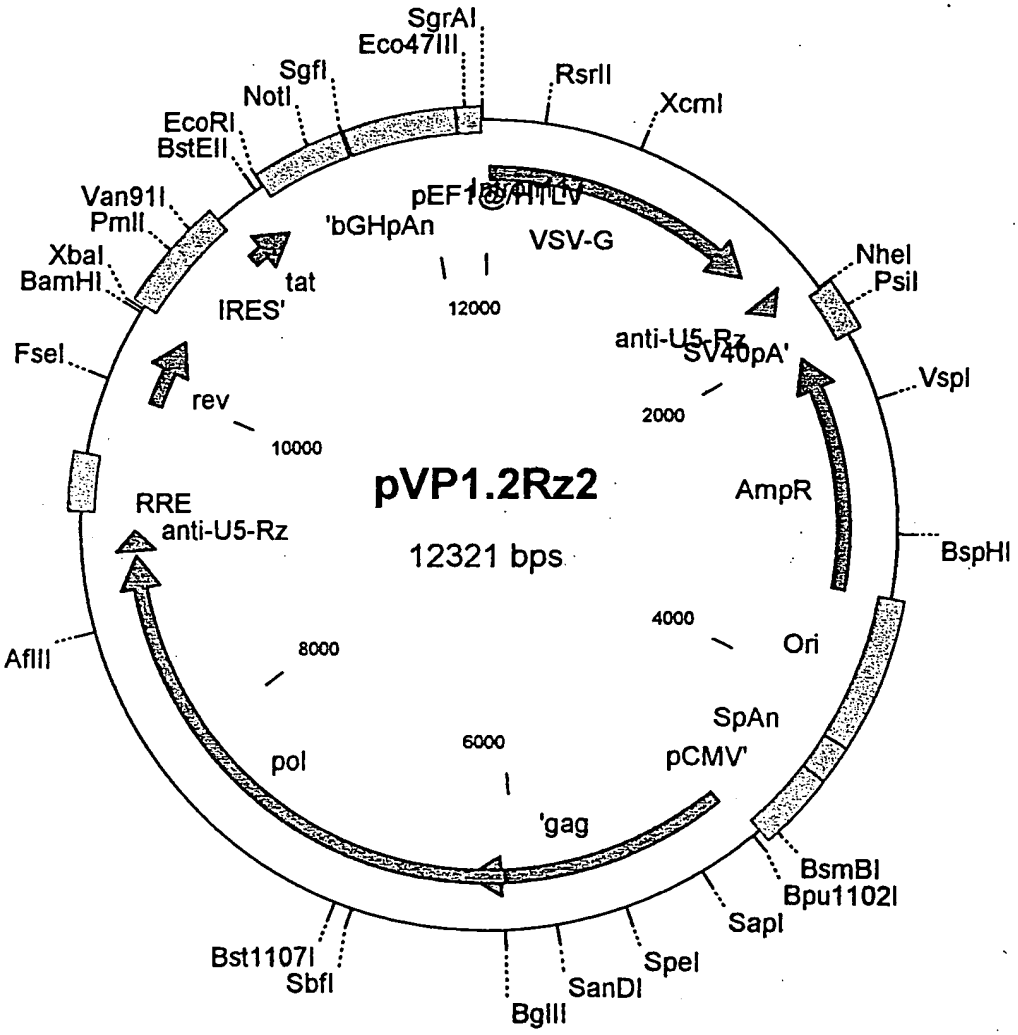

pVP1.2Rz2
12321 bps
SgrAI
Eco47III
SgfI
NotI
EcoRI
BstEII
Van91I
PmlI
XbaI
BamHI
FseI
AflII
Bst1107I
SbfI
BglII
SanDI
SpeI
SapI
BsmBI
Bpu1102I
BspHI
VspI
NheI
PsiI
RsrII
XcmI
'bGHpAn
tat
IRES'
rev
RRE
anti-U5-Rz
pol
'gag
pCMV'
SpAn
Ori
AmpR
anti-U5-Rz
SV40pA'
VSV-G
2000
4000
6000
8000
10000
12000

Fig 6G

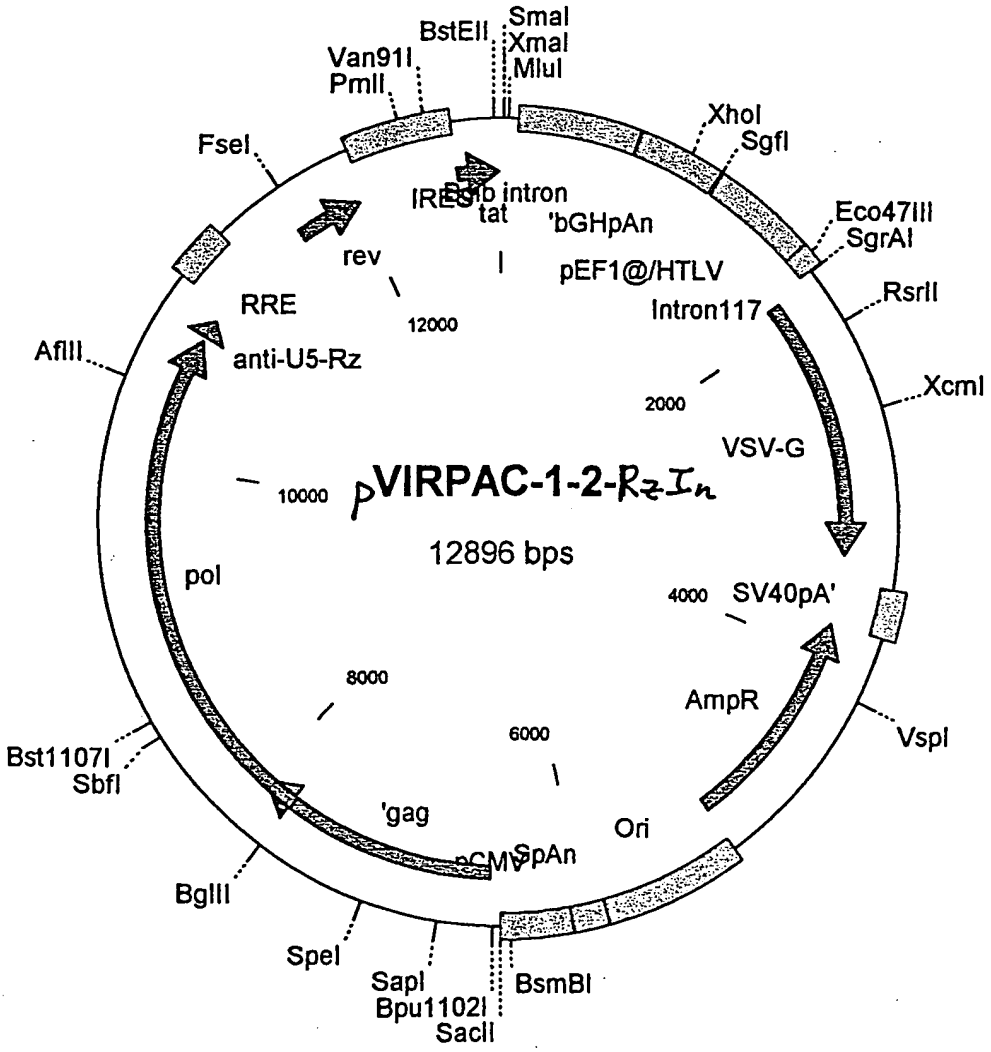

pVIRPAC-1-2-RzIn
12896 bps
SmaI
XmaI
MluI
BstEII
Van91I
PmlI
FseI
XhoI
SgfI
Eco47III
SgrAI
RsrII
XcmI
VsplI
AflII
Bst1107I
SbfI
BglII
SpeI
SapI
Bpu1102I
SacII
BsmBI
rev
RRE
anti-U5-Rz
IRES
tat
'bGHpAn
pEF1@/HTLV
Intron117
VSV-G
SV40pA'
AmpR
Ori
'gag
pol
12000
2000
4000
6000
8000
10000

Fig 7

Influence of Ribozyme(s) in the Packaging on pN1(cPT)GFP Vector Titers in HeLa-tat Cells

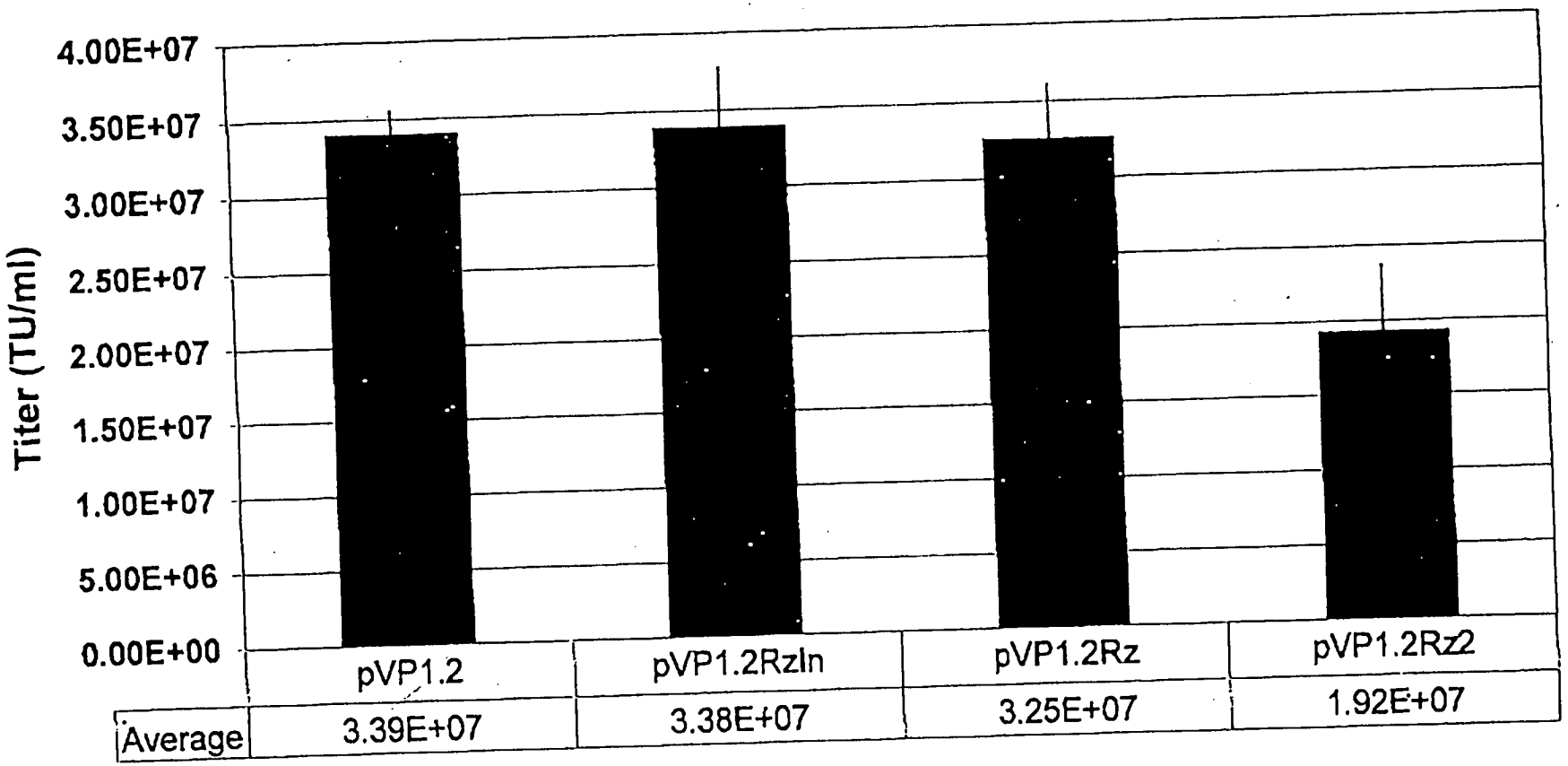


Fig 8

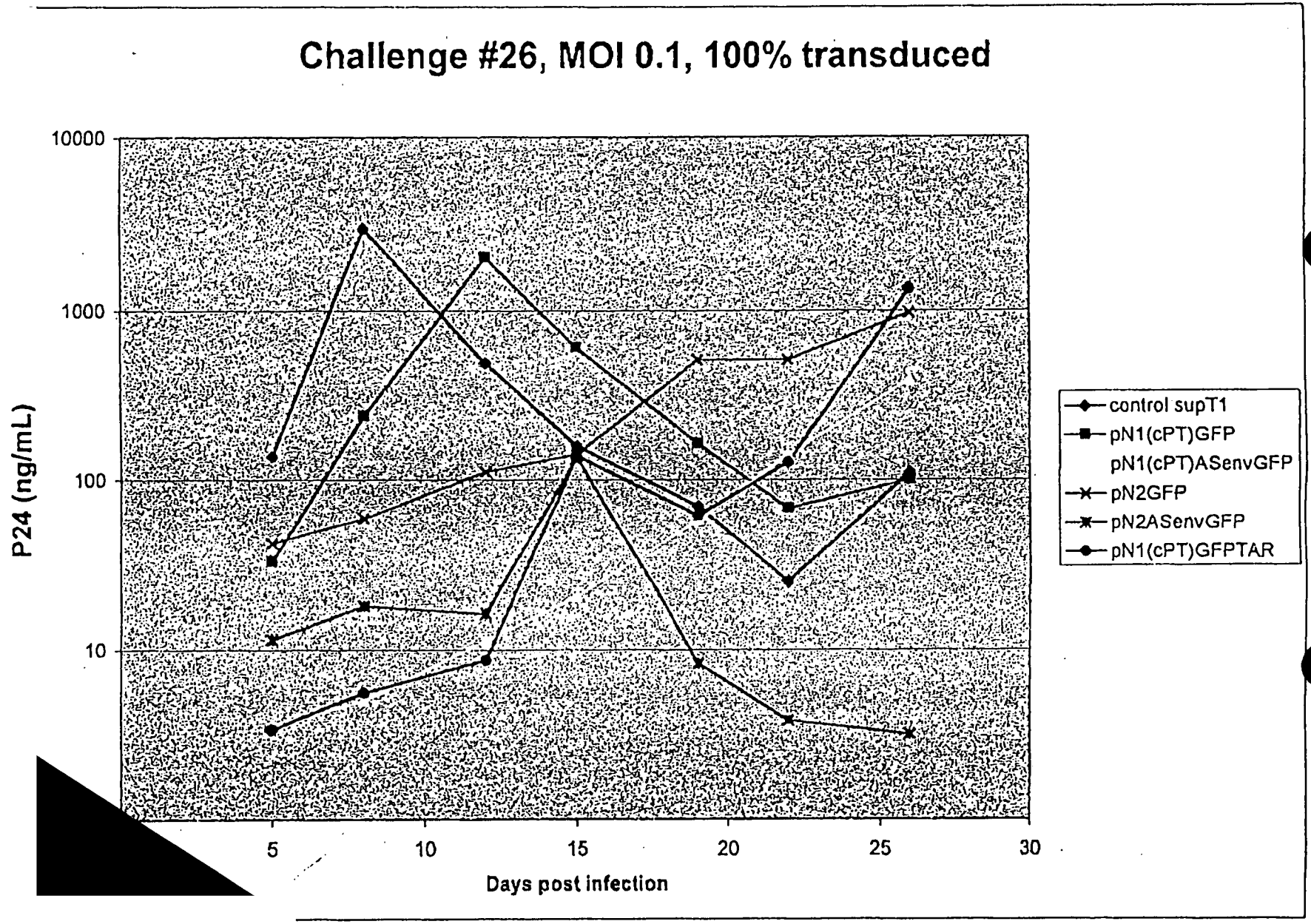

Challenge #26, MOI 0.1, 100% transduced
P24 (ng/mL)
10000
1000
100
10
5
10
15
20
25
30
Days post infection
control supT1
pN1(cPT)GFP
pN1(cPT)ASenvGFP
pN2GFP
pN2ASenvGFP
pN1(cPT)GFPTAR

FIGURE 9A

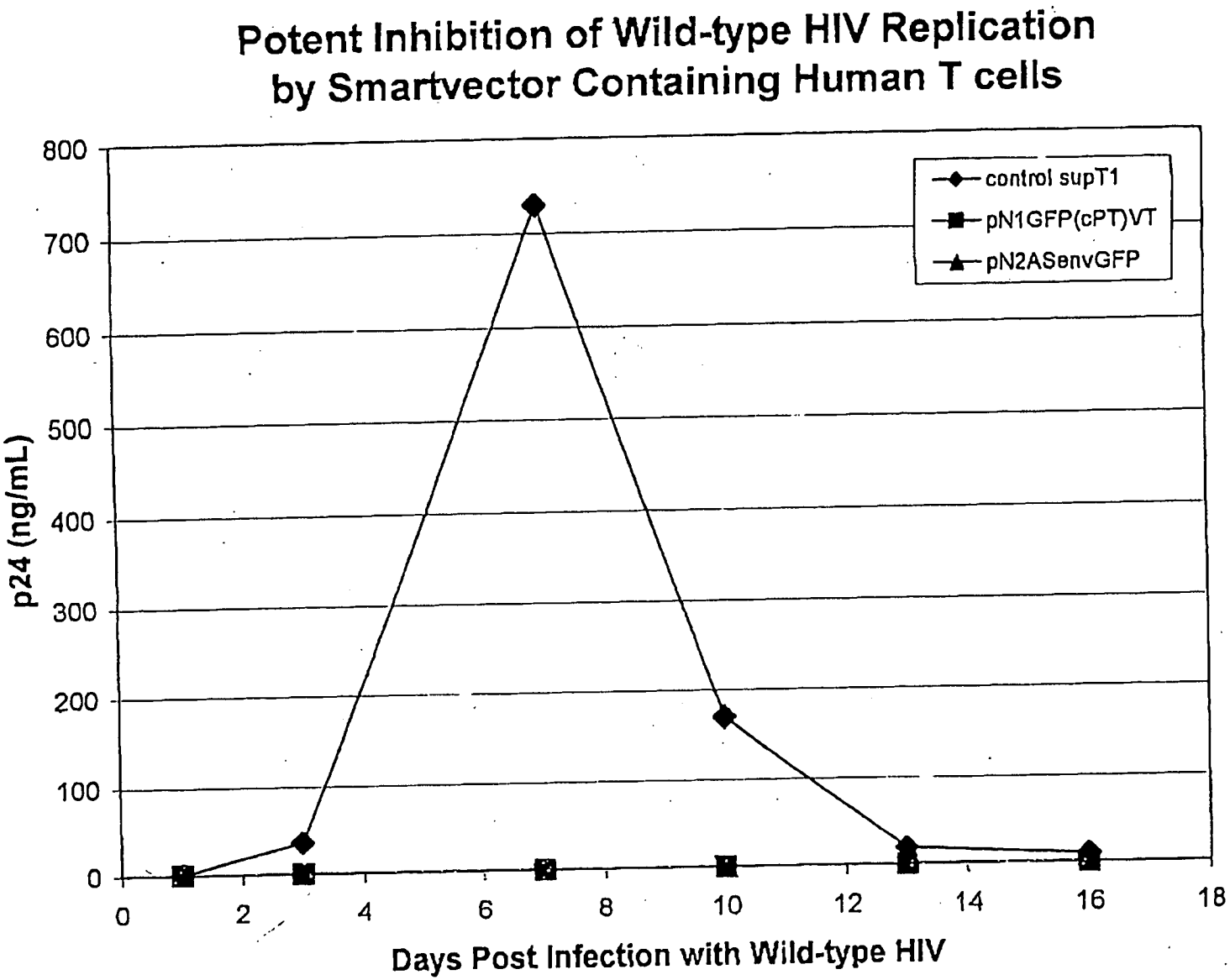


FIGURE 9B

Potent Inhibition of Wild-type HIV Replication by Smartvector Containing T Cells

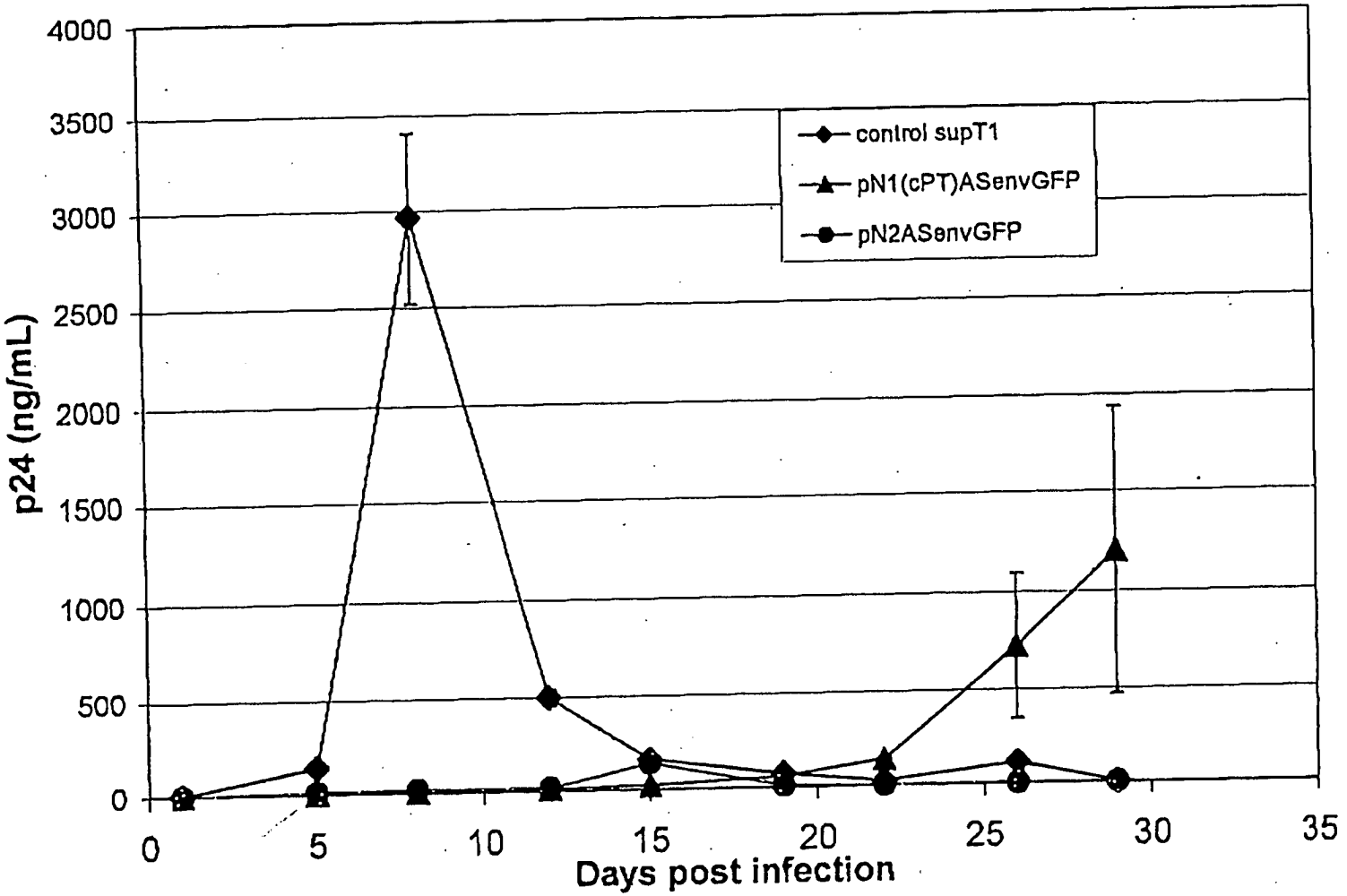
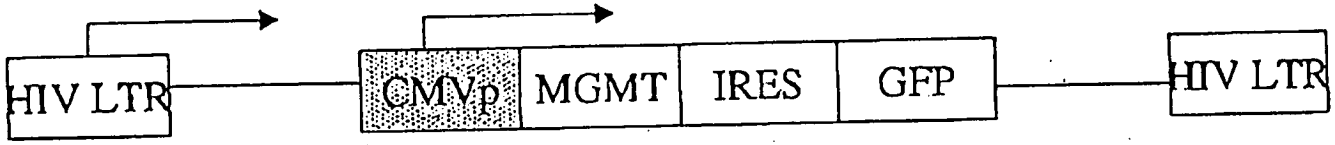
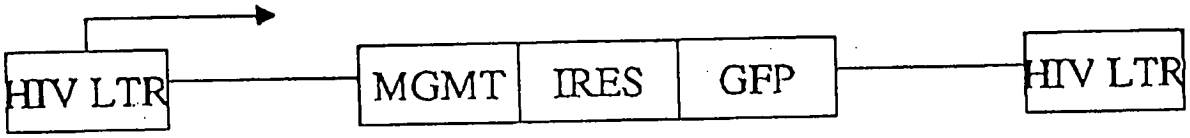


Fig 10A

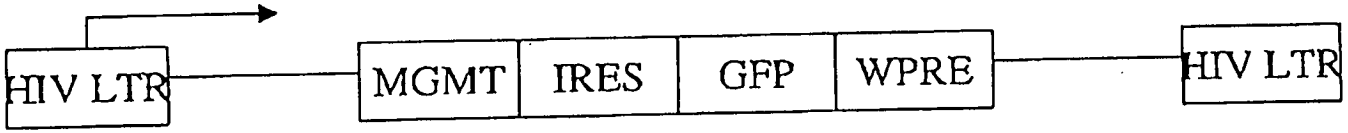
pN1CMIG



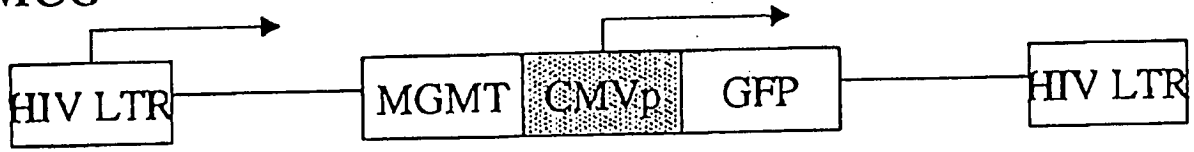
pN1MIG



pN1MIG-W



pN1MCG



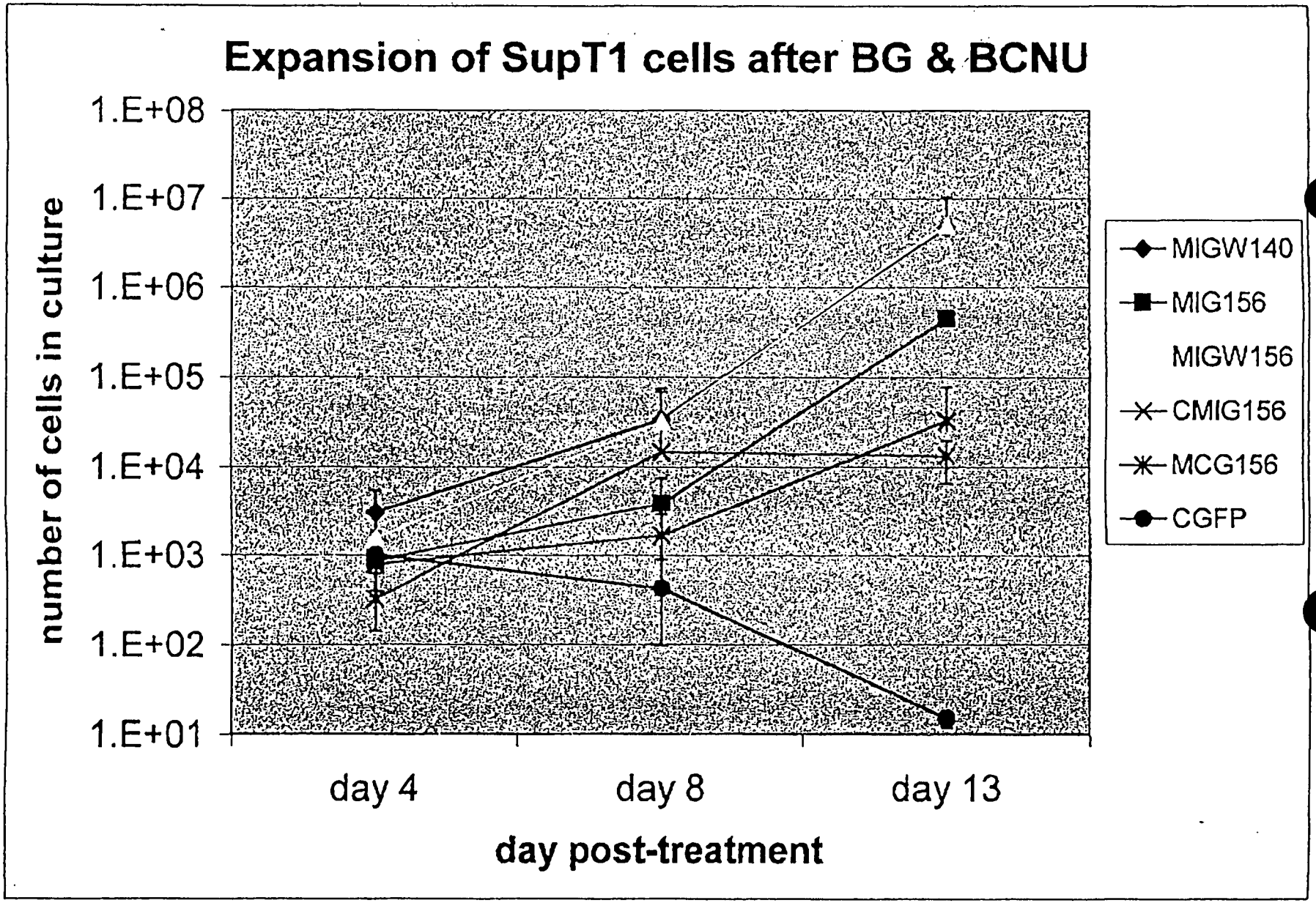
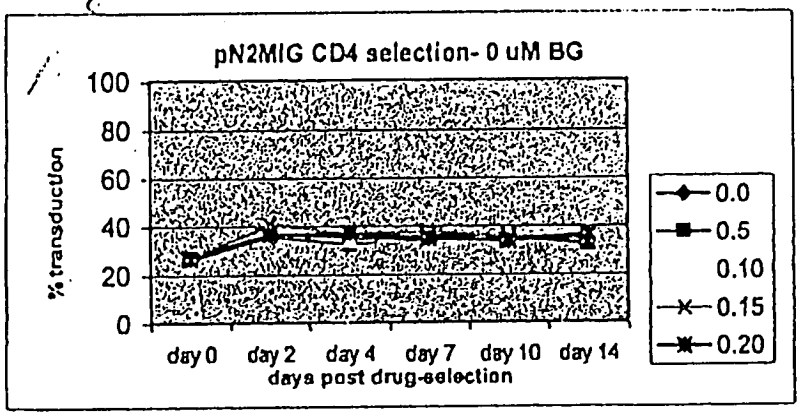
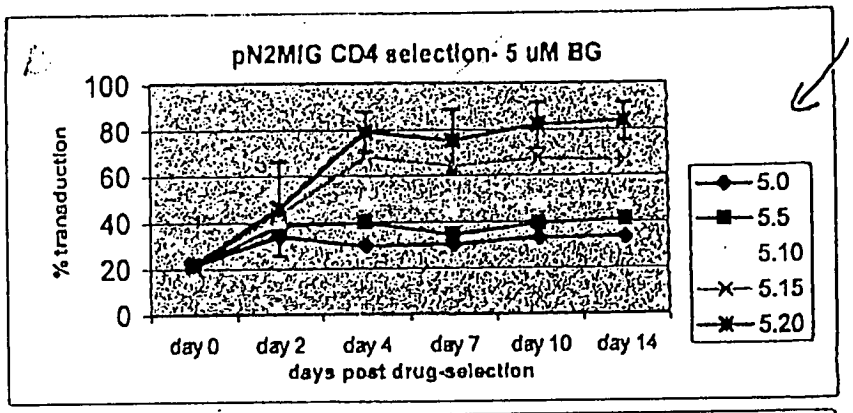
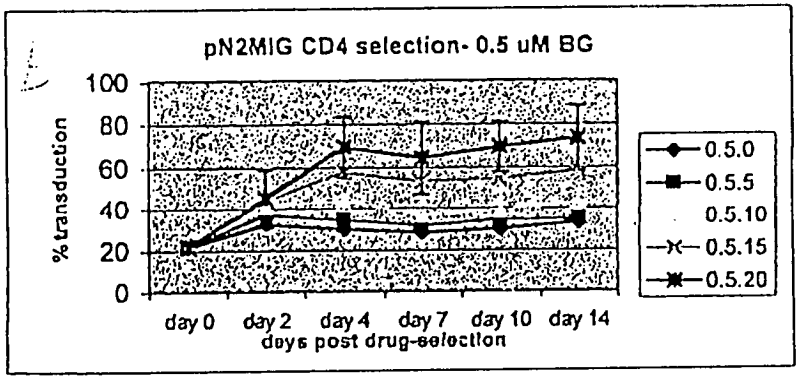
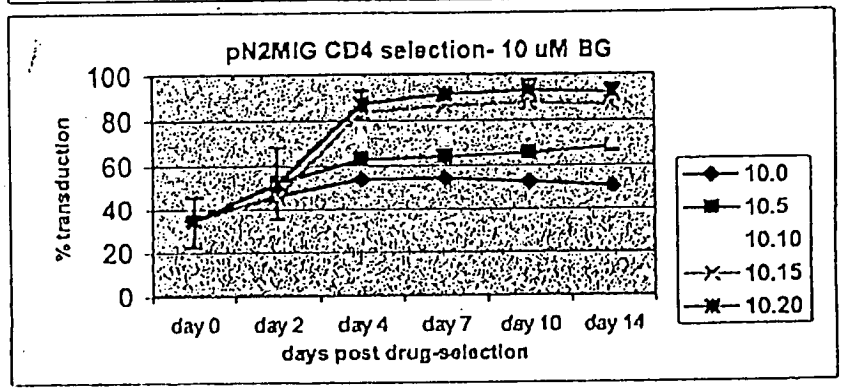

Expansion of SupT1 cells after BG & BCNU
number of cells in culture
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
day 4
day 8
day 13
day post-treatment
MIGW140
MIG156
MIGW156
CMIG156
MCG156
CGFP

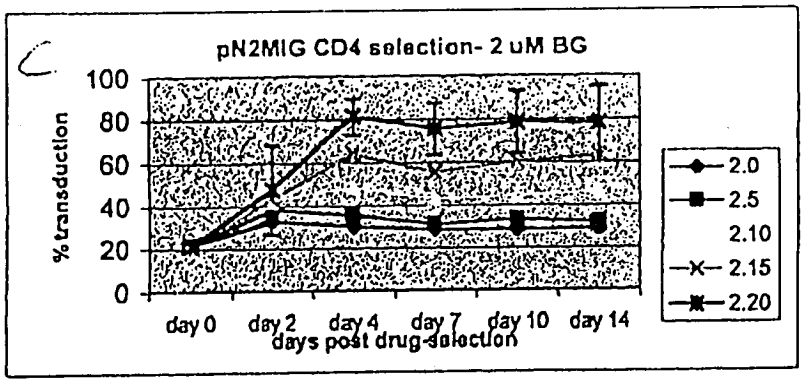
Fig 11

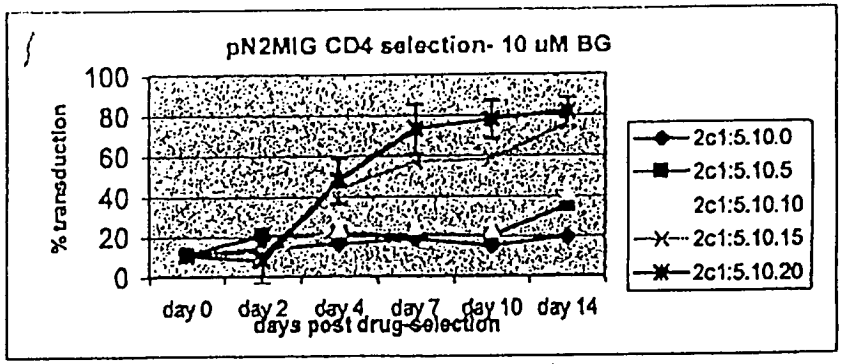

pN2MIG CD4 selection- 0 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
0.0
0.5
0.10
0.15
0.20

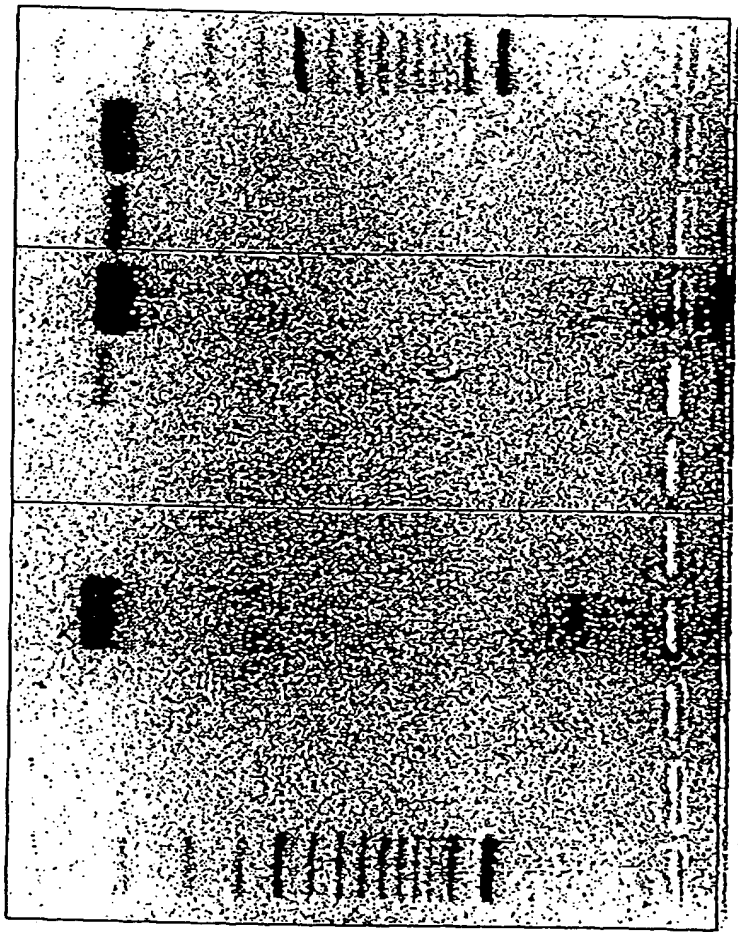

pN2MIG CD4 selection- 5 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
5.0
5.5
5.10
5.15
5.20


pN2MIG CD4 selection- 0.5 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
0.5.0
0.5.5
0.5.10
0.5.15
0.5.20


pN2MIG CD4 selection- 10 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
10.0
10.5
10.10
10.15
10.20

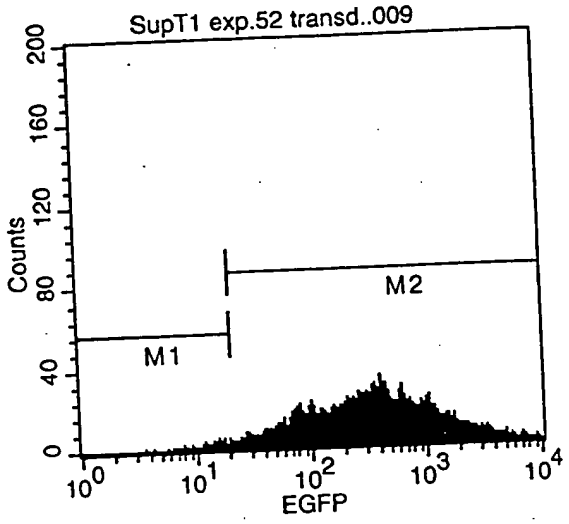

pN2MIG CD4 selection- 2 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
2.0
2.5
2.10
2.15
2.20


pN2MIG CD4 selection- 10 uM BG
% transduction
100
80
60
40
20
0
day 0
day 2
day 4
day 7
day 10
day 14
days post drug-selection
2c1:5.10.0
2c1:5.10.5
2c1:5.10.10
2c1:5.10.15
2c1:5.10.20



Marker
1 pN1 CGFP 1C exp 30
3 pN1 CGFP 2C exp 30
1-4 pVP1.2
9-12 pVP1.2 Rz
13-16 pVP1.2 Rz2
pNL4-3 with DNase I
pNL4-3 without DNase I
Amp. Neg. Control
Extraction Neg. Control
Marker

Fig 13A



Histogram Statistics

File: SupT1 exp.52 transd..009
Tube: pN1(cPT)ASenvGFP 452 a

Sample ID: SupT1 ex
Acquisition Date: 25-

Marker	Left, Right	Events	% Gated	% Total	Mean
All	1, 9910	6356	100.00	63.56	570.39
M1	1, 20	95	1.49	0.95	13.86
M2	20, 9910	6262	98.52	62.62	578.74

Fig 13A

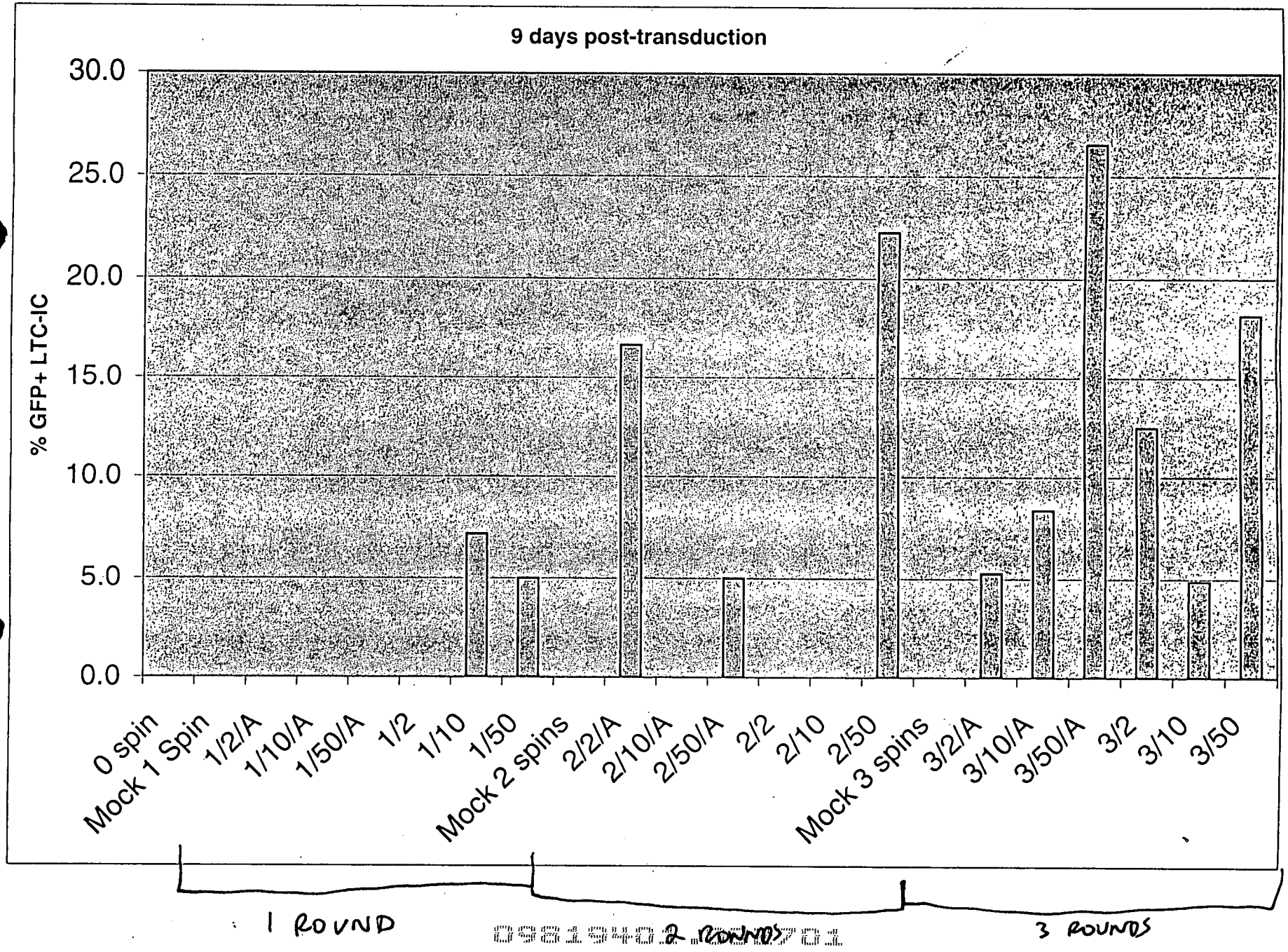

9 days post-transduction
% GFP+ LTC-IC
30.0
25.0
20.0
15.0
10.0
5.0
0.0
0 spin
Mock 1 Spin
1/2/A
1/10/A
1/50/A
1/2
1/10
1/50
Mock 2 spins
2/2/A
2/10/A
2/50/A
2/2
2/10
2/50
Mock 3 spins
3/2/A
3/10/A
3/50/A
3/2
3/10
3/50
1 ROUND
2 ROUNDS
3 ROUNDS

Fig 14A

Vsv-G, RD114 AND RD114-VSV-G CHIMERIA ENVELOPE PROTEINS

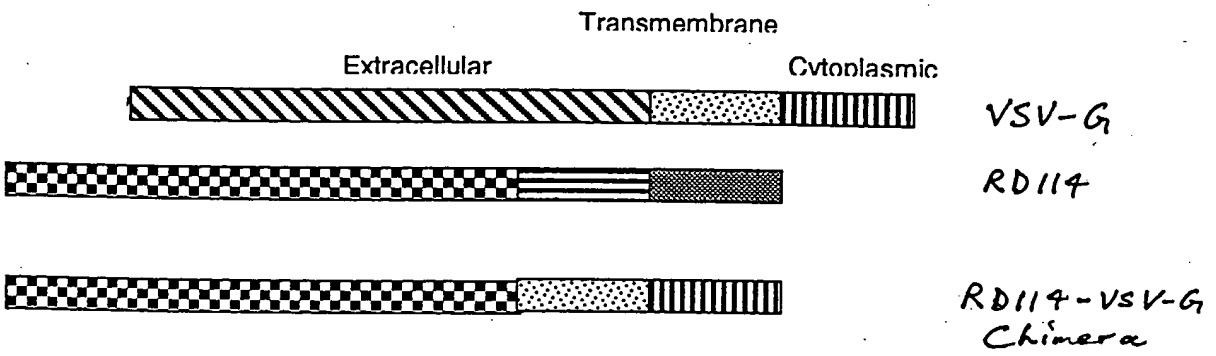


Fig 14B

Titers of RD114-pseudotyped HIV-1 vectors in HT1080

Envelopes	IU/ml
VSV G	3.5x10e6
Rabies virus G	1.6x10e6
RD114WT env	1.5x10e5
RD114E env	3.8x10e4

Fig 15A

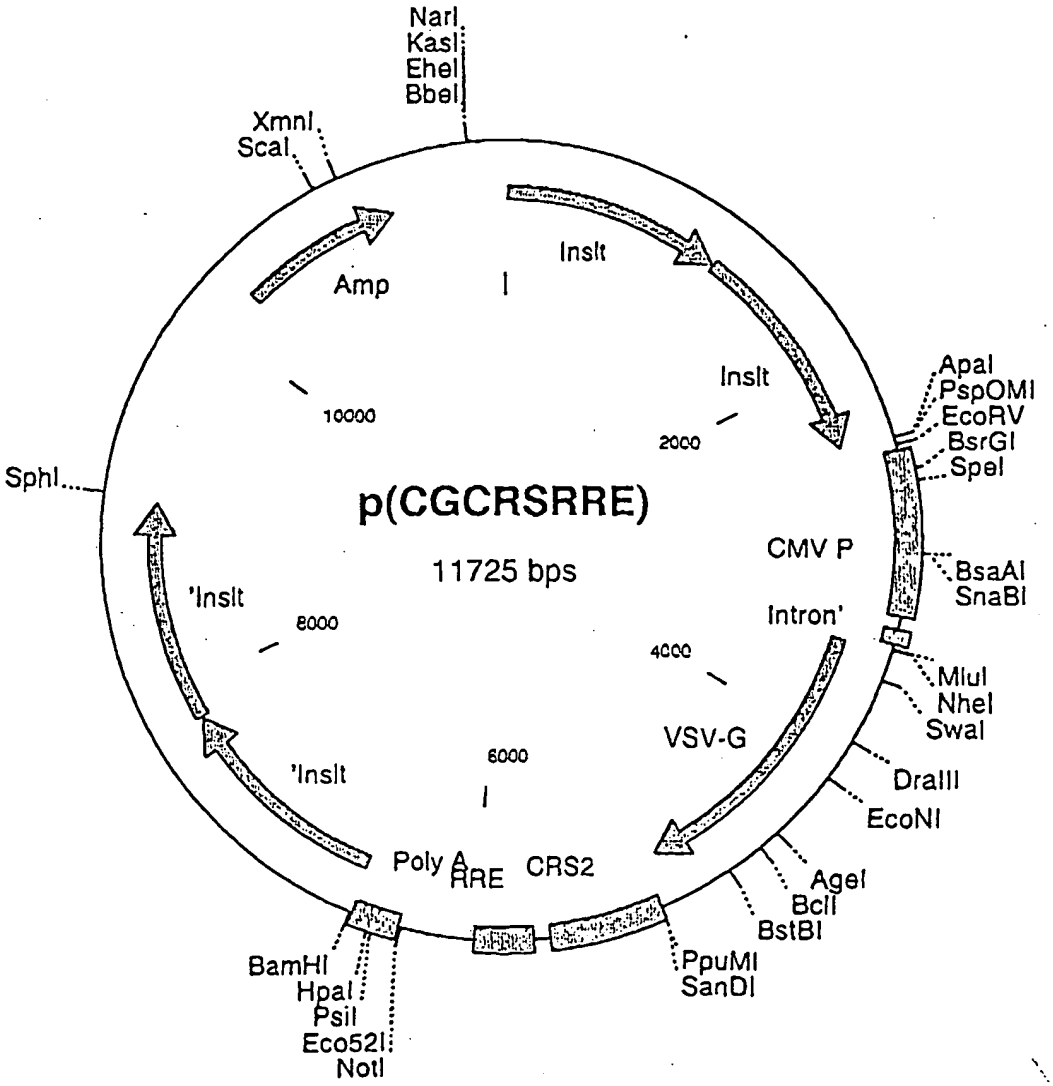

NarI
KasI
EheI
BbeI
XmnI
ScaI
SphI
Amp
InsIt
InsIt
'InsIt
'InsIt
10000
2000
8000
4000
6000
p(CGCRSRRE)
11725 bps
CMV P
Intron'
VSV-G
Poly A
RRE
CRS2
ApaI
PspOMI
EcoRV
BsrGI
SpeI
BsaAI
SnaBI
MluI
NheI
SwaI
DraIII
EcoNI
AgeI
BclI
BstBI
PpuMI
SanDI
BamHI
HpaI
PsiI
Eco52I
NotI

Fig 15A

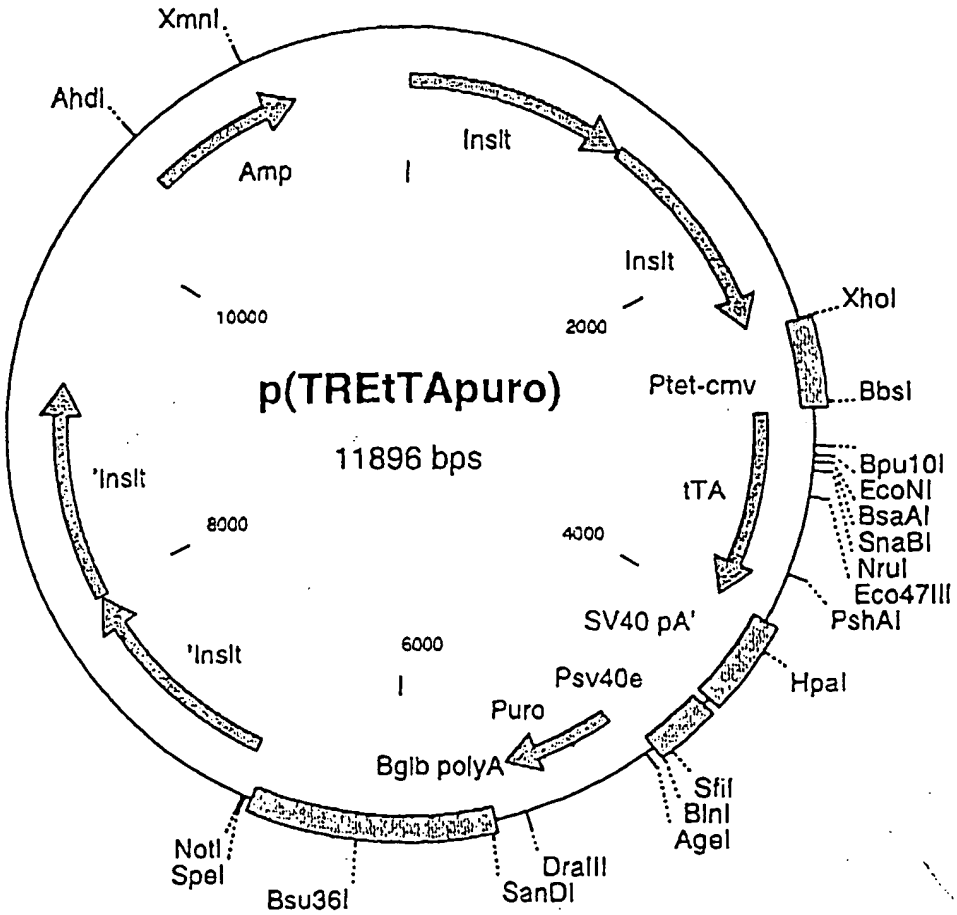

p(TREtTApuro)
11896 bps
Xmnl
Ahdl
Amp
InsIt
InsIt
Xhol
BbsI
Bpu10I
EcoNI
BsaAI
SnaBI
NruI
Eco47III
PshAI
HpaI
Ptet-cmv
tTA
SV40 pA'
Psv40e
Puro
BglI polyA
SfiI
BlnI
AgeI
DraIII
SanDI
Bsu36I
NotI
SpeI
'InsIt
'InsIt
10000
2000
4000
6000
8000

Fig 15C

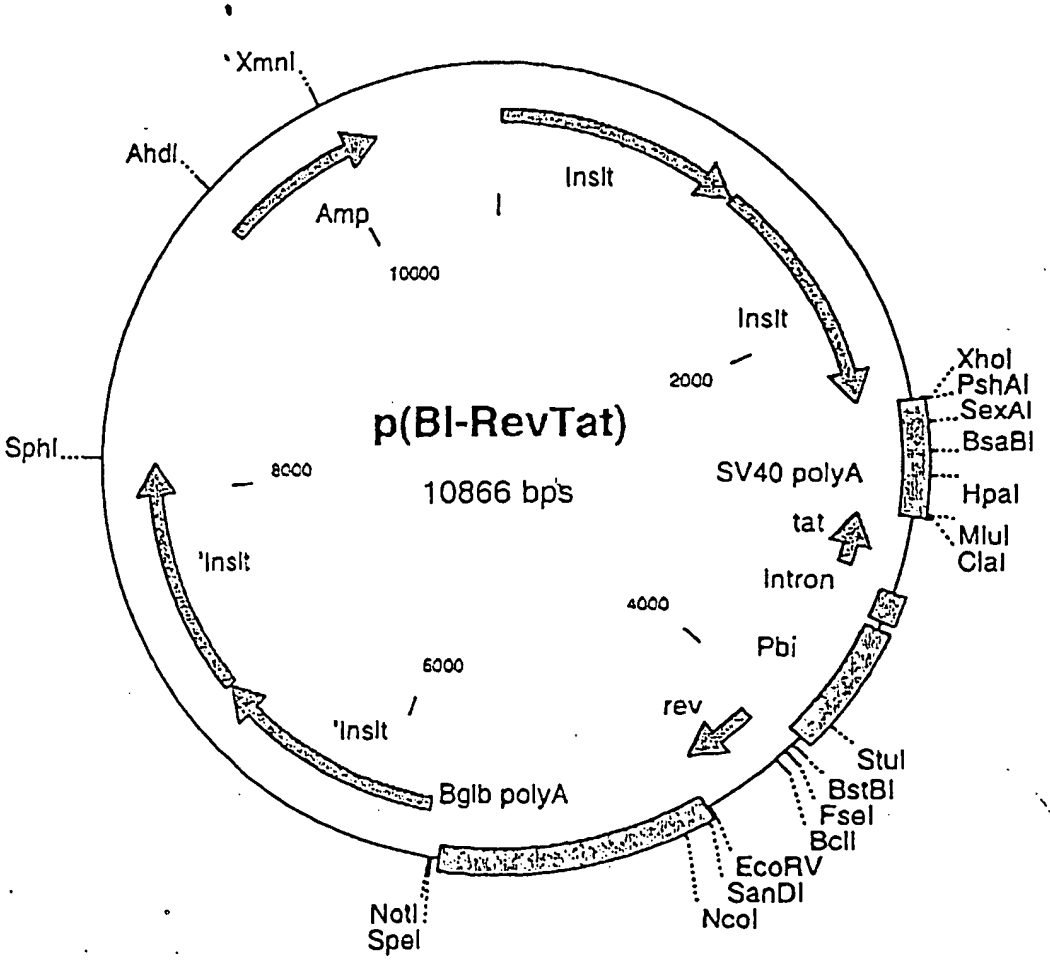

Xmnl
Ahdl
Amp
10000
InsIt
2000
InsIt
p(Bl-RevTat)
10866 bps
SV40 polyA
tat
Intron
4000
Pbi
rev
6000
'InsIt
'InsIt
8000
SphI
Bglb polyA
NotI
SpeI
StuI
BstBI
FseI
BclI
EcoRV
SanDI
NcoI
XhoI
PshAI
SexAI
BsaBI
HpaI
MluI
ClaI

Fig 15D

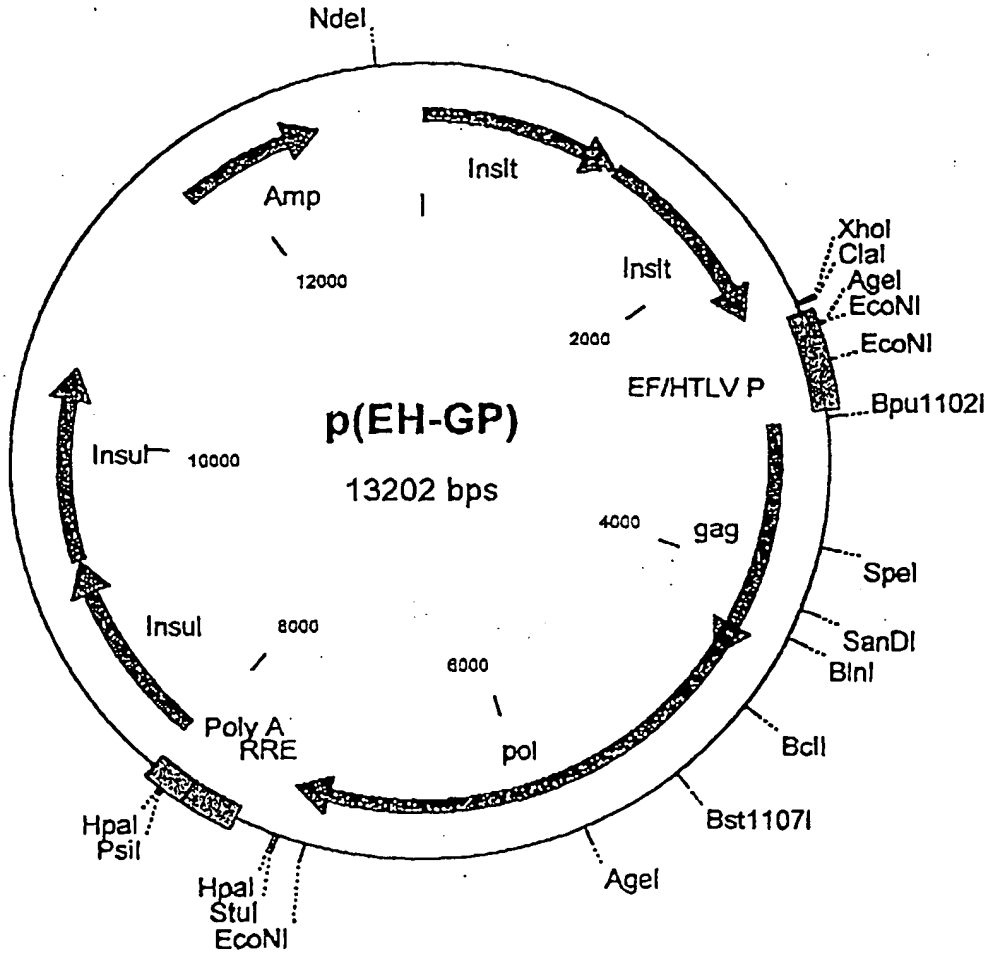

NdeI
InsIt
Amp
12000
InsIt
2000
XhoI
ClaI
AgeI
EcoNI
EcoNI
Bpu1102I
EF/HTLV P
p(EH-GP)
13202 bps
4000
gag
SpeI
SanDI
BlnI
BclI
Bst1107I
AgeI
pol
6000
8000
Insul
Poly A
RRE
HpaI
PsiI
HpaI
StuI
EcoNI
Insul
10000

Fig 15E

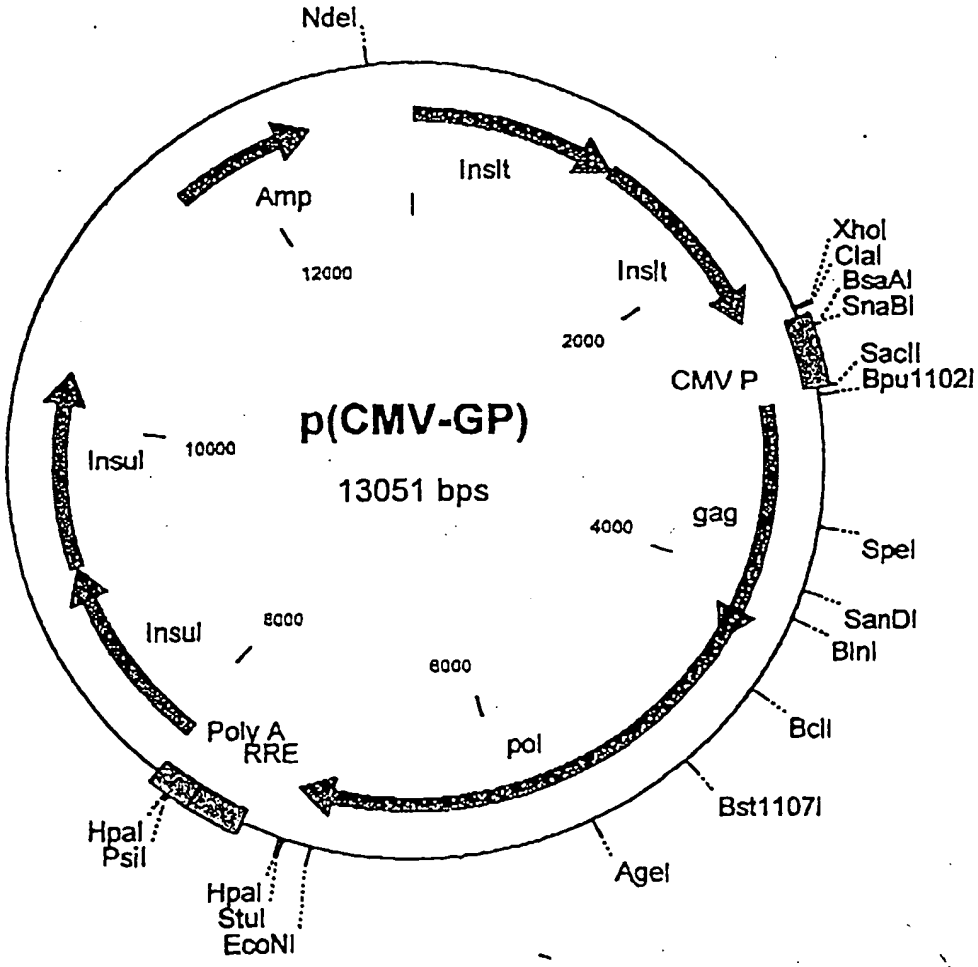

Ndel
InsIt
Amp
12000
InsIt
2000
XhoI
ClaI
BsaAI
SnaBI
SacII
Bpu1102I
CMV P
p(CMV-GP)
13051 bps
10000
Insul
4000
gag
SpeI
SanDI
BlnI
Insul
8000
6000
BclI
Poly A
RRE
pol
Bst1107I
HpaI
PsiI
AgeI
HpaI
StuI
EcoNI

Fig 15F

Rev dependent VSV-G constructs

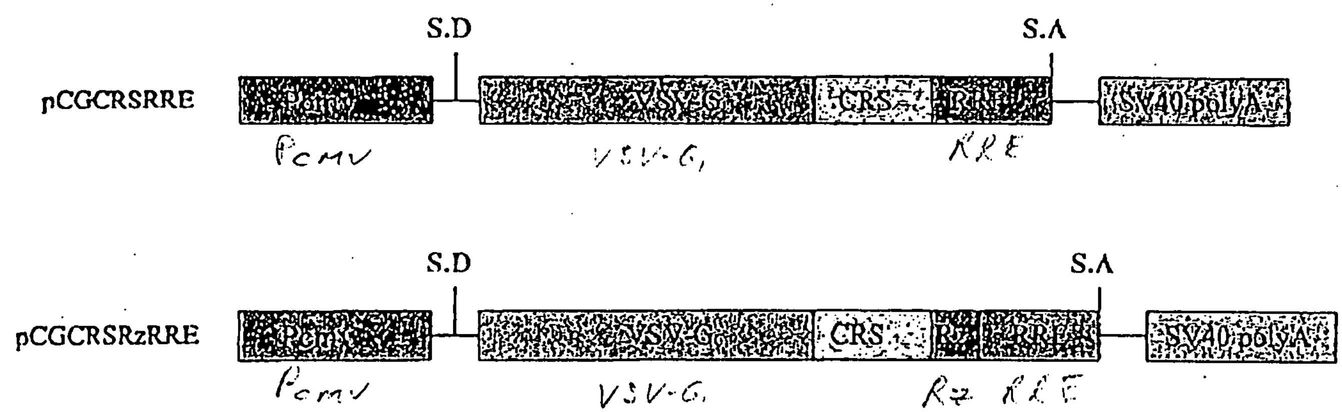
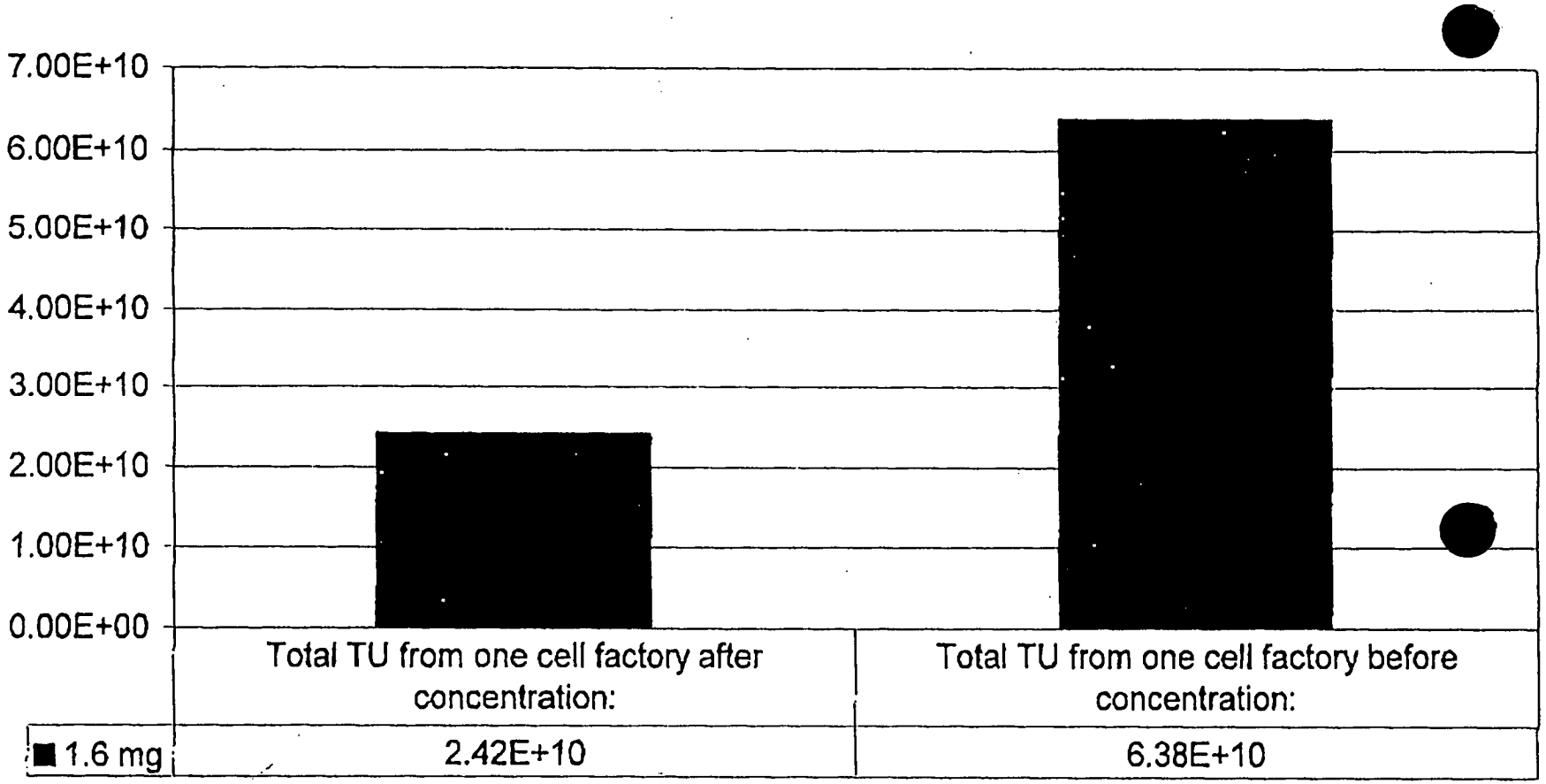


Figure 2

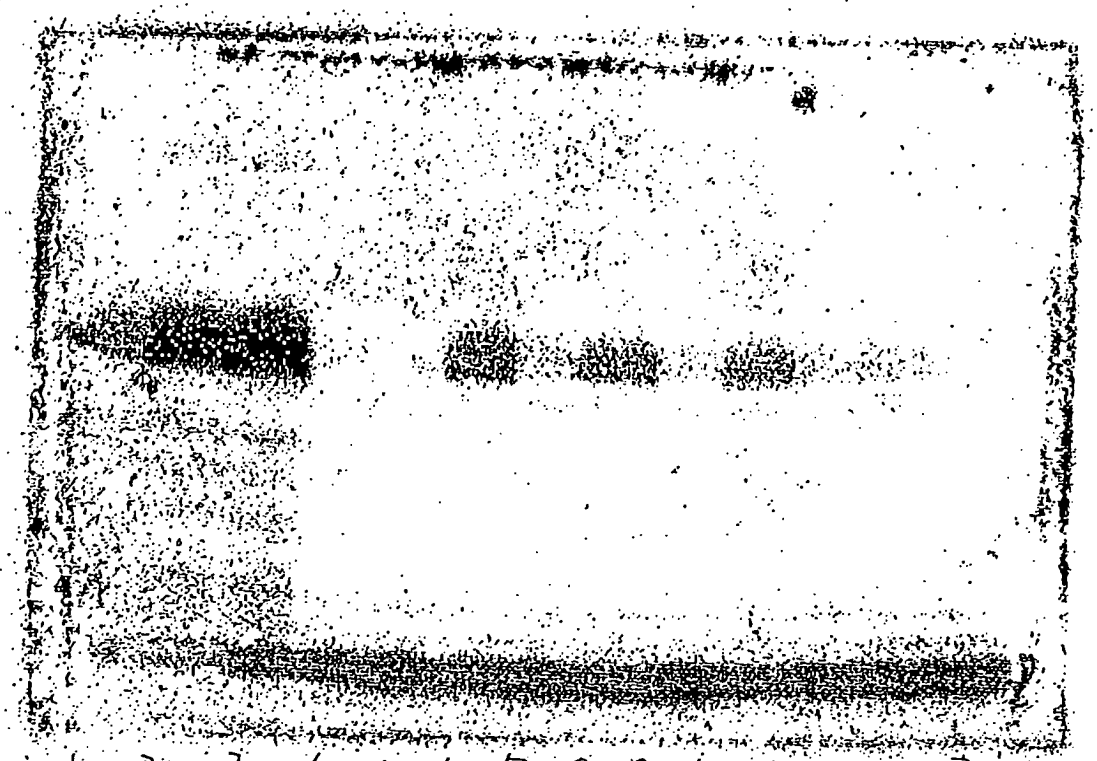
Yeild of pN1(cPT)GFP Vectors per Cell Factory before and after Concentration in HeLa-tat Cells.



AFTER CONCENTRATION

BEFORE CONCENTRATION.

Fig 17



1 2 3 4 5 6 7 8 9 10 11 12 13

TETRACYCLINE + − + − + − + − + − + −

293G

LANE X 3 5 4

pCMV-VSVG

pCGRS-RRE-G

pCGRSRRE-1M

pCGRSRRE-H

pCGRSRRE-1E

pCGRS-RRE-2E

+ : pCMV-Rev

− : pCI

G : β-globin SD

1M — HIV-1 major SD

H — Hammarskjold's SD Analog

1E — HIV-1 env SD

2E — HIV-2 env SD

REMOVE TETRACYCLINE TO INDUCE EXPRESSION OF VSV-G THAT IS REV DEPENDENT.

Fig 18

Influence of the Buffer on Vector Recovery after Storage for 3-5 Weeks at Different Temperatures

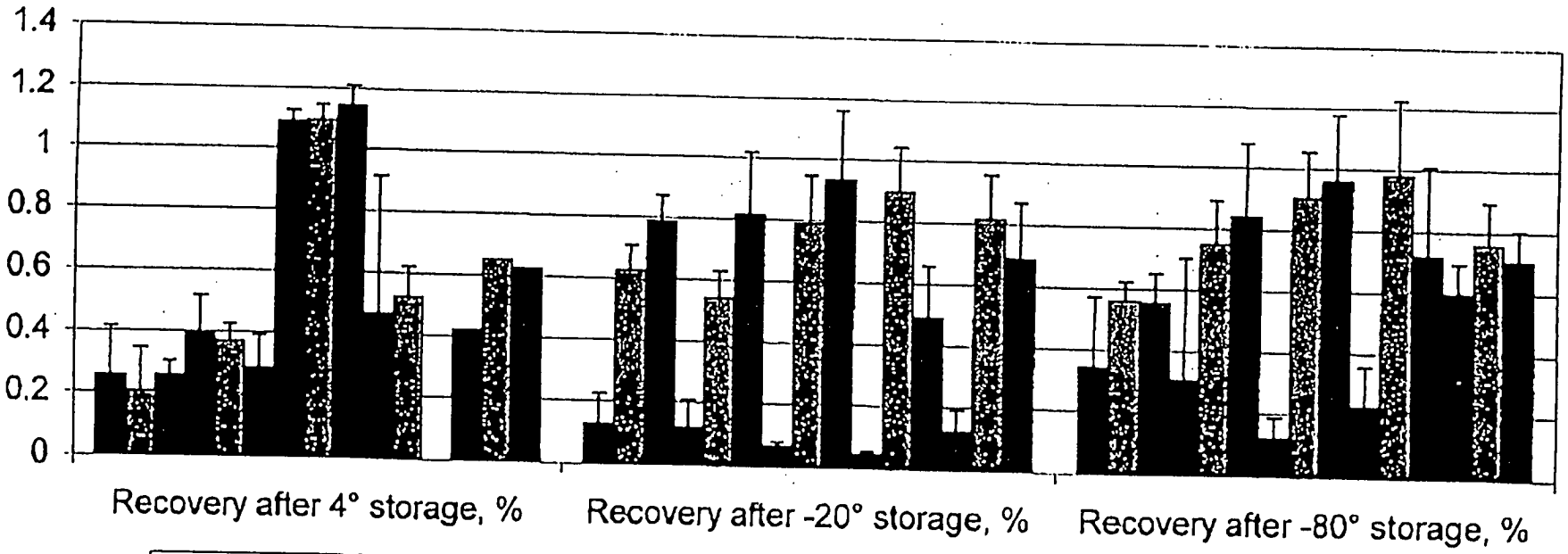
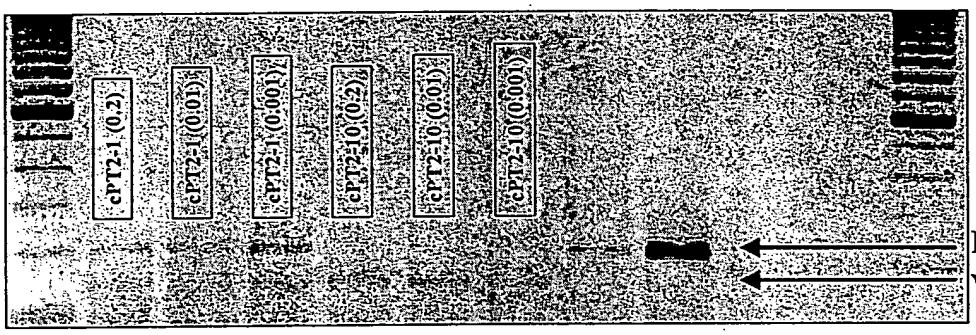


Figure 19


cPT2-1 (0.2)
cPT2-1 (0.01)
cPT2-1 (0.001)
cPT2-10 (0.2)
cPT2-10 (0.01)
cPT2-10 (0.001)
HIV wt
Vector